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OBSERVATIONS ON CAPILLARY AND VENOUS BLOOD IN TRAUMATIC SHOCK

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THE simplicity of technique and apparatus and the speed of results have given the recently described copper sulfate[1] method for determining hematocrits and plasma proteins a well-deserved popularity. This method was used routinely in our evacuation hospital as a guide in the treatment of the shock in war wounds and in the controlled fluid therapy in pre- and postoperative patients.

Cannon and associates[2] in World War I noted a disproportionately high erythrocyte count in capillary as compared with venous blood in cases of wound shock. Based on this, the authors of the copper sulfate method caution against using capillary blood specific gravity in cases of shock. The suggestion is made that differences in specific gravity between capillary and venous blood might be used to diagnose the shut down of peripheral circulation. This implication is also made by Scudder and Self.[3]

Using the copper sulfate method in the manner to be described, simultaneous capillary and venous whole blood and serum gravities were done on seventy-seven war casualties in varying degrees of secondary shock. The comparative results proved of no value in the diagnosis of shock; in many instances capillary and venous blood serum can be used interchangeably. This communication is based on the seventy-seven cases.

METHOD

All capillary and venous gravity determinations were done by one individual. The venous blood was drawn from the antecubital vein whenever accessible and transferred to a tube containing 1 mg. ammonium-potassium oxalate mixture per cubic centimeter of added blood; 3 c.c. were allowed to clot in a test tube for serum.

Capillary blood was obtained at the same time; whenever accessible, the ear lobe was used. The skin was cleansed, dried, and punctured deeply with a lancette. The blood was drawn into a pipette and dropped immediately into the copper sulfate solutions. Any dropper with a moderately fine point can be used. Approximately ten drops of capillary blood were then collected in capsules for serum. Small test tubes, as suggested by Kagan,[4] were first used but were soon discarded for the capsules described by Lyon.[5] Satisfactory receptacles were made using glass tubing with an inside diameter of 3 mm.

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The length of the entire capsule was approximately 7 cm., with a capillary end about 1 cm. in length, and the "blowhole" was about 4 cm. from the closed end. There is very little difficulty in filling this tube if it is shaken down between drops. The blood was allowed to clot, the capillary end broken, and the clot separated from the walls. The open end was sealed with adhesive tape, placed in a corked centrifuge tube, and spun down.

For measuring specific gravities of whole blood, solutions graded at intervals of 0.004 were found sufficiently accurate. In the plasma range, solutions graded at intervals of 0.002 were used. In comparing whole blood gravities, 0.0005 was subtracted from the venous blood value to correct for the added anticoagulant. This was also subtracted from the venous plasma.

The procedure employed for the formol-gel test was essentially comparable to that used by most others. Two drops of 36 per cent formalin were added to 1 c.c. of serum, and one drop of formalin was added to 1 c.c. of plasma. The tubes were mixed by inversion, corked, and left at room temperature for twelve hours. Degrees of viscosity were estimated by tilting the tubes. Positive reactions were designated by the numbers 1 to 4, ranging from a slight change in viscosity to a solid gel.

RESULTS

Comparisons between capillary and venous blood in sixteen normal subjects are listed in Table I. In general, the comparative values for whole blood and hematocrits are in fairly close agreement. The first study on simultaneous capillary and venous serum protein was apparently made by Kagan, using the rate of fall of a drop of serum as a measure of its protein content. In fourteen normal adults and two children he found no difference more than 0.2 Gm. per 100 c.c. With our technique the capillary serum value is often

TABLE I. COMPARISON OF CAPILLARY AND VENOUS BLOOD IN NORMAL SUBJECTS

PATIENT	WHOLE BLOOD* SPECIFIC GRAVITY		SERUM PROTEINS (GM./100 C.C.)		PLASMA PROTEINS* (GM./100 C.C.)	CALCULATED HEMATOCRIT† (PER CENT)	
	VENOUS	CAP.	VENOUS	CAP.		VENOUS	CAP.
1	1.0605	1.060	7.0	6.8	7.2	47	47
2	1.0595	1.059	6.2	6.3	6.3	48	47
3	1.0575	1.058	6.2	6.5	6.3	45	45
4	1.059	1.060	6.3	6.6	6.5	47	48
5	1.0585	1.061	6.2	6.2	6.7	47	50
6	1.059	1.060	7.3	7.3	7.3	45	46
7	1.060	1.060	6.2	6.2	6.7	49	49
8	1.061	1.062	6.8	7.2	7.3	49	49
9	1.0595	1.059	6.5	7.2‡	7.2	47	45
10	1.0595	1.059	7.2	7.1	7.3	46	45
11	1.058	1.058	6.7	6.8	7.0	45	45
12	1.0565	1.056	6.5	6.7	6.7	43	42
13	1.0565	1.057	5.8	6.0	6.3	45	45
14	1.0585	1.058	6.3	6.7	6.5	46	45
15	1.0625	1.066	6.6	7.8§	7.0	51	54
16	1.0575	1.057	6.0	6.2	6.0	46	45

*0.0005 has been subtracted for the anticoagulant.
†In calculating the hematocrit from the line chart, the serum protein value was used.
‡Serum leaked out while centrifuging.
§Great deal of squeezing necessary; serum hemolyzed.

more than 0.2 Gm. higher. This might be explained by the difference in the manner of blood collection. Lowry and Hunter[6] have recently compared serum specific gravity of venous blood with finger and ear blood and have found no significant difference in normal individuals.

Abnormally high capillary serum protein values will be obtained if the capillary tube is not sealed and the centrifuge tube not tightly corked during centrifugation. Excessive squeezing with hemolysis of the capillary blood usually results in high protein values. To measure the effect of squeezing the ear or finger on the capillary whole blood specific gravity, the weights of the first drops of blood obtained after puncturing were compared with the weight of drops obtained after the capsule had been filled. The average fall in specific gravity in the sixteen normal subjects was 0.002.

Examples of each type of injury causing traumatic shock are recorded and grouped in Table II. These include extremity wounds with extensive soft tissue laceration, extremity wounds with hemorrhage or fracture, chest wounds with hemothorax, abdominal wounds, combined abdominal and chest wounds, crush injuries, burns, and head wounds. The findings in the remainder of the seventy-seven cases studied are, for the purposes of this report, essentially the same. There is no consistent or significant difference between capillary and venous whole blood gravity. Any discrepancy fits into the normal range and can usually be accounted for by the error in the method.

The two cases of generalized edema included in Table II were studied to obtained the effect of edema on the capillary blood gravity. It is apparent from these as well as from the traumatic cases with generalized edema due to hypoproteinemia that the effect on the capillary values is not above the error in the method.

In fifty battle casualities the effect of squeezing on the capillary blood gravity was, in general, the same as in the normal subjects. The average decrease was 0.002 in drops collected before and after filling the capillary capsule, irrespective of the degree of shock or the presence of edema.

It was fully realized that the total serum protein did not indicate the proportion of albumin and globulin. Thus a hypoproteinemia due to a low albumin fraction would be concealed by a rising globulin concentration, the total protein being normal. There is no accepted simple rapid quantitative test for determination of albumin or globulin separately. The only alternative was to use a semiquantitative test for hyperglobulinemia. The serum formol-gel reaction is not strictly correlative with the serum globulin, yet it has been found to be positive in varying degrees in sera containing more than 3.5 Gm. globulin per 100 c.c.[7-10] In over 250 determinations on seventy-seven severely wounded soldiers, many of whom were checked from eight to ten days after injury, the reaction was positive (1 plus) on only three occasions.

The normal difference between the plasma and serum protein, which is apparently due mainly to fibrinogen, averages 0.4 Gm. per 100 c.c. of plasma (Table I). The danger of using this factor to interchange plasma and serum

TABLE II. CAPILLARY AND VENOUS BLOOD STUDIES

PATIENT	DATE OF INJURY	TIME OF INJURY	TYPE OF INJURY	TIME BLOOD COLLECTED	B/P	PULSE	GENERAL CONDITION AND COMMENT	CONDITION OF SKIN AND EDEMA	PREVIOUS AND FLUID
R. H.	10/8	2 P.M.	Traumatic amputation and extensive muscle evulsion, right lower arm and shrapnel wound right thigh	10/8 6 P.M.	60/40	140	Poor	Pale, cool	None
				10/9 9 A.M.	120/60	106	Fair	Warm, pink	Amputation arm 10/8 c.c. United and 1,000 No I.V. th
				10/10 9:30 A.M.	120/78	108	Fair	Warm, pink	
H. H.	10/9	8 A.M.	Perforated wound left ankle, thigh, calf, buttock, and right foot, with muscle evulsion	10/10 10 A.M.	95/45	160	Poor	Warm	Debridemen 10/10, 2 plasma a United S
				10/11 9:30 A.M.	118/70	160	Poor; delirious	Warm, moist	Debridemen 10/10 9 United S
				10/12 10 A.M.	112/70	160	Poor; delirious	Warm	500 c.c. pla
H. C.	12/1	8 P.M.	Traumatic amputation left foot, with compound comminuted fracture left leg and penetrating wound right leg and thigh	12/5 11 A.M.	88/40	140	Poor	Warm, pink	1,000 c.c. P.M.; am thigh an wounds 500 c.c. 1,000 c.c. blood No I.V. the
				12/6 9 A.M.	80/40	120	Poor	Warm, pink	
C. L.	1/1	6 A.M.	Penetrating wound leg and thigh, with compound comminuted fracture femur; lacerated penis and scrotum; penetrating wound arm and right thigh	1/1 1 P.M.	42/35	124	Bad	Pale, cool	0 c.c. pla 7:30 A.M.
				1/2 10 A.M.	75/45	132	Poor	Pinker, warm	500 c.c. bl c.c. plasm tion thigh 1/2, 1:30
Y. B.	1/2	8 P.M.	Land mine; compound comminuted fracture both ankles and destruction right foot	1/3 3 P.M.	70/50	160	Bad; irrational	Pale, moist	000 c.c. bl c.c. plasm
G. W.	10/12	1 P.M.	Gunshot wound left ankle, thigh, and penis; superficial wound back	10/12 6 P.M.	80/60	110	Poor	Pale, warm	No I.V. the
				10/13 9:30 A.M.	105/70	88	Good	Warm, pink	Debridement 10/12, 10 c.c. blood 000 c.c. pla c.c. United
J. H.	11/21	--	Shrapnel wounds both legs with compound comminuted fracture tibia; exposed three days	11/25 11:45 A.M.	50/30	100	Bad; unconscious; died 12/25, 1:30 P.M.	Pale	
F. W.	11/30	3 A.M.	Land mine; multiple wounds arm, ankles, and forearm	11/30 11 A.M.	140/70	160	Bad	Warm, pink	500 c.c. plasm
H. V.	12/4	12 M.	Gunshot wounds both thighs with compound fracture left femur	12/4 7 P.M.	0/0	120	Bad; unconscious; died 12/4 11 P.M.	Pink, cool	1,500 c.c. pla 1,000 c.c. blood

*The United States blood contained 500 c.c. of anticoagulant solution to 500 c.c. of blood.

†Opales

PREVIOUS TREATMENT AND FLUID THERAPY	WHOLE BLOOD SPECIFIC GRAVITY		SERUM PROTEIN (GM. PER CENT)		PLASMA PROTEIN (GM. PER CENT)	PLASMA FORMOL-GEL	CALCULATED HEMATOCRIT (PER CENT)	
	VENOUS	CAP.	VENOUS	CAP.			VENOUS	CAP.
one	1.0505	1.053	4.8	5.8	5.5	0	39	40
mputation at right upper arm 10/8, 11 P.M.; 2,000 c.c. United States* blood and 1,000 c.c. plasma	1.0405	1.0405	5.5	5.3	5.6	0	23	24
o I.V. therapy	1.0415	1.041	5.5	5.5	5.6	0	25	24
ébridement some wounds, 10/10, 2 A.M.; 500 c.c. plasma and 2,000 c.c. United States blood	1.0505	1.0495	4.6	4.8	5.0	0	39	37
ébridement other wounds, 10/10 9 P.M.; 1,000 c.c. United States blood	1.0465	1.046	4.8	4.8	5.3	2	34	33
00 c.c. plasma	1.0445	1.044	5.0	5.1	5.5	4	30	29
,000 c.c. plasma 12/4, 12 P.M.; amputation left thigh and débridement wounds 12/5, 6 A.M.; 500 c.c. plasma, and 1,000 c.c. United States blood	1.0435	1.0445	5.1	5.5	5.3	0	29	29
o I.V. therapy	1.0405	1.040	4.9	4.8	5.3	0 Opales.†	25	25
0 c.c. plasma, 1/1, 7:30 A.M.	1.0405	1.041	5.1	5.3	6.0	0 Opales.	24	25
,500 c.c. blood and 900 c.c. plasma; amputation thigh and testicle, 1/2, 1:30 A.M.	1.0395	1.038	5.1	5.3	5.6	0	23	21
,000 c.c. blood and 1,500 c.c. plasma	1.048	1.049	5.8	6.0	6.0	0	33	34
o I.V. therapy	1.051 R.B.C., 3.4	1.052 R.B.C., 3.55	5.0	5.5	5.3	0	39	39
ébridement wounds, 10/12, 10 P.M.; 1,000 c.c. blood	1.048	1.046	5.1	5.0	5.3	0	35	32
,000 c.c. plasma and 2,000 c.c. United States blood	1.0435	1.043	5.6	5.8	6.2	0 Opales.	27	26
00 c.c. plasma at 5 A.M.	1.0445	1.044	6.0	6.0	6.5	0 Opales.	27	27
,500 c.c. plasma and 1,000 c.c. United States blood	1.0395	1.039	5.3	5.4	5.6	0	22	22

†Opalescent.

TABLE II

PATIENT	DATE OF INJURY	TIME OF INJURY	TYPE OF INJURY	TIME BLOOD COLLECTED	B/P	PULSE	GENERAL CONDITION AND COMMENT	CONDITION OF SKIN AND EDEMA
T. J.	12/4	8 A.M.	Shrapnel wound left hand	12/5 7 P.M.	50/0	120	Bad; congestive failure	Warm, pink, with ankle edema
				12/6 9 A.M.	100/64	100	Poor	Warm, pink
W. H.	4/11	8 P.M.	Gunshot wound buttock with compound fracture ileum	4/12 4:30 P.M.	60/0	120	Bad	Pale
R. E.	10/8	10 A.M.	Shrapnel wound upper arm with lacerated brachial artery and vein	10/8 3:30 P.M.	94/58	128	Poor	Pale, cool
				10/9 9 A.M.	128/82	116	Good	Warm, pink
C. R.	11/25	7 P.M.	Shrapnel wounds of both thighs and scrotum, with lacerated femoral vein	11/26 3 P.M.	90/55	124	Poor	Pale; no edema
				11/26 7 P.M.	75/40	128	Poor	Pale; no edema
				11/27 9:30 A.M.	100/65	104	Fair	Warm
				11/28 11:30 A.M.	100/60	100	Fair	Warm
M. F.	4/12	12 M.	Gunshot wound thigh with lacerated femoral vein and scrotum	4/12 4:30 P.M.	70/40	80	Bad	Pale
				4/13 10 A.M.	105/60	72	Poor	Pinker
K. J.	11/8	4 A.M.	Shell wound left chest with hemothorax	11/10 9 A.M.	150/100	120	Fair	Warm
				11/11 8:30 A.M.	130/90	128	Fair	Warm
M. J.	12/8	--	Shell wound chest and multiple wounds knee with compound comminuted fracture of femur	12/16 12 M.	120/80	120	Poor	Pale
				12/17 3 P.M.	140/88	88	Fair	Pinker
H. J.	12/31	10:30 A.M.	Shrapnel wound abdomen with lacerated jejunum	12/31 2:30 P.M.	80/65	84	Bad	Warm, moist, pink
				12/31 9 P.M.	135/80	88	Fair	Warm, dry, pink
D. W.	11/30	3 A.M.	Land mine; multiple perforations of intestine; traumatic amputation right foot	11/30 11 A.M.	100/40	126	Bad	Warm, pale
				12/2 2 P.M.	135/70	108	Poor	Moist, pink; died 12/11

—CONT'D

PREVIOUS TREATMENT AND FLUID THERAPY	WHOLE BLOOD SPECIFIC GRAVITY		SERUM PROTEIN (GM. PER CENT)		PLASMA PROTEIN (GM. PER CENT)	PLASMA FORMOL-GEL	CALCULATED HEMATOCRIT (PER CENT)	
	VENOUS	CAP.	VENOUS	CAP.			VENOUS	CAP.
Amputation two fingers, 12/4, 6 P.M.	1.0495	1.050 Finger 1.052 Ear	5.1	5.1	5.6	0	37	37
750 c.c. plasma; three ampules of digitalis	1.0515	1.052 Finger 1.053 Ear	6.2	6.3	6.5	0 Opales.	37	37
No I.V. therapy	1.0515	1.051	6.2	6.5	6.7	0 Opales.	37	35
600 c.c. plasma, 10/8, 11 P.M.	1.0485	1.047	5.6	5.5	5.6	0	34	38
Débridement of wounds and ligation brachial artery and vein; 1,000 c.c. United States blood	1.0445	1.045	4.8	4.8	5.0	0	31	32
1,000 c.c. blood and 1,000 c.c. glucose and saline; débridement wounds, and ligation femoral vein, 11/26, 9 A.M.	1.043	1.042	3.8	3.8	4.3	0	32	31
No I.V. therapy	1.043	1.042 Finger and ear	3.8	3.9	4.1	0	32	30
1,000 c.c. blood and 1,000 c.c. plasma	1.0455	1.0455	5.1	5.4	5.6	0 Opales.	32	31
Amputation right knee, 11/27	1.0445	1.044	5.1	5.2	5.6	4	30	29
1,000 c.c. of plasma, 12/4, 2 P.M.	1.0415	1.039	5.5	5.5	5.6	0	25	22
1,000 c.c. United States blood, 500 c.c. plasma, and 1,000 c.c. glucose and saline	1.0385	1.038	5.1	5.1	5.6	0	22	21
Aspiration of 150 c.c. blood, 11/8; 1,000 c.c. United States blood, and 1,000 c.c. glucose and saline	1.058	1.056	5.8	6.0	6.7	4	47	44
1,000 c.c. glucose and saline; drinking freely	1.056	1.055	5.5	5.3	6.3	4	45	44
Thoracotomy, 12/8, and débridement of leg	1.0495	1.050	5.3	5.6	6.2	4	36	36
Amputation at thigh, 12/16; 1,000 c.c. blood, 1,000 c.c. glucose and saline, and 500 c.c. plasma	1.0505	1.050	4.8	5.1	5.6	4	39	38
No I.V. therapy	1.061	1.061	6.2	6.8	6.4	0	50	49
500 c.c. plasma and 1,000 c.c. United States blood	1.061	1.060	6.2	6.8 Hemolyzed	7.2	0	50	47
1,000 c.c. plasma, 5 A.M.	1.045	1.044	5.8	6.0	6.3	0	29	27
Amputation at hip; peritonitis; 4,000 c.c. glucose and saline, 1,000 c.c. blood, and 250 c.c. plasma, 12/1	1.0515	1.0485	5.3	5.3	6.0	4	39	35

TABLE II

PATIENT	DATE OF INJURY	TIME OF INJURY	TYPE OF INJURY	TIME BLOOD COLLECTED	B/P	PULSE	GENERAL CONDITION AND COMMENT	CONDITION OF SKIN AND EDEMA
D. A.	12/26	--	Shell wound chest into abdomen through spinal cord	11/28 11:30 A.M.	95/65	132	Poor	Warm, pink
				11/28 7 P.M.	90/50	132	Poor; died 12/4	Moist; jaundiced
C. R.	12/29	2 P.M.	Shell wound fracturing kidney, through liver, into lung with pneumohemothorax	12/30 10 A.M.	125/100	108	Poor	Pale, moist, warm
				12/31 10 A.M.	145/85	140	Poor	Edema of ankles
R.B.	10/21	9 A.M.	Vehicle overturned; crush injury chest with fractured humerus	10/21 10 A.M.	80/60	140 (Irreg.)	Poor	Warm, pink
				10/21 2 P.M.	30/20	140	Poor	Warm, pink; died, 3 P.M.
M. W.	11/7	3 P.M.	Crushed between wall and truck; multiple rib fracture	11/7 6:30 P.M.	70/40	140	Poor	Pale, cold
				11/8 8:30 A.M.	100/60	120	Fair	Warm
M. H.	10/11	8 A.M.	Second-degree burns of 62 per cent body surface	10/12 9:30 P.M.	100/80	124	Poor	Edematous
				10/13 10 A.M.	118/80	128	Poor	Edematous
				10/13 8:30 P.M.	128/60	138	Poor	Edematous
K. E.	4/16	4 A.M.	Second-degree burns, legs, hand, face; 45 per cent body surface	4/16 10:30 A.M.	100/80	112	Bad	Little leg edema
				4/17 9 A.M.	90/70	120	Poor	Little leg edema
				4/18 9:30 A.M.	90/60	124	Poor	Moderate leg edema
M. S.	4/16	4 A.M.	Second-degree burns face, wrist, thigh, and buttock; 30 per cent body surface	4/16 11 A.M.	125/85	64	Poor	--------
				4/17 9 A.M.	100/65	60	Fair	No edema
H. N.	11/24	7:30 A.M.	Compound comminuted fracture frontal bone with evisceration and lacerated brain with extensive hemorrhage	11/24 10 A.M.	85/50	80	Bad; unconscious	Pale
D. R.			Nephrosis	5/9	170/110			Edema face, hands, and legs, and ascites
				5/13				Edema face, hands and legs with ascites
W. R.			German prisoner for eight months; nutritional deficiency					Edema face, hands and legs with ascites

—CONT'D

PREVIOUS TREATMENT AND FLUID THERAPY	WHOLE BLOOD SPECIFIC GRAVITY		SERUM PROTEIN (GM. PER CENT)		PLASMA PROTEIN (GM. PER CENT)	PLASMA FORMOL-GEL	CALCULATED HEMATOCRIT (PER CENT)	
	VENOUS	CAP.	VENOUS	CAP.			VENOUS	CAP.
Aspiration of chest, 11/26; 1,000 c.c. blood, 11/27; splenectomy, 11/28	1.0545	1.054	5.5	5.8	6.3	1	43	41
No I.V. therapy	1.0575	1.0575	6.0	6.1	6.5	4	46	45
Thoracotomy and laparotomy, 12/30, 2 A.M., 3,000 c.c. blood at operation	1.055	1.053	5.5	5.8	5.7	0	43	40
3,000 c.c. glucose and saline	1.060	1.061	5.3	5.8	6.0	1	50	51
No I.V. therapy	1.0565	1.0535	5.4	5.6	5.3	0	46	41
500 c.c. plasma and 500 c.c. blood; autopsy showed hematoma of chest and back muscles	1.048	1.0485	5.1	5.1	5.4	0	34	35
350 c.c. plasma	1.0515	1.051	5.5	5.5	5.6	0	38	38
150 c.c. plasma and 1,000 c.c. United States blood	1.050	1.051	5.1	5.1	5.3	0	37	38
4,000 c.c. plasma; local débridement	1.0645	1.064 Toe	--	6.2 Squeezed hard	5.6	0 Opales.	--	54
1,500 c.c. plasma	1.0605	1.061 Toe	5.6	5.8	6.2	4	50	51
1,000 c.c. plasma	1.0555	1.053 Toe	5.5	5.6	6.5	4	44	41
1,000 c.c. plasma	1.0645	1.064	6.2	7.2 Centrifuge leaked	7.0	0	55	52
1,500 c.c. plasma and 1,000 c.c. 5 per cent glucose; local débridement	1.0625	1.064	5.8	6.2	6.0	0 Opales.	53	54
1,000 c.c. plasma; drinking freely	1.0605	1.059	4.8	5.1	5.7	4	52	49
500 c.c. plasma	1.0555	1.059 Ear	6.5	7.9 Excessive squeezing	7.0	0	42	44
Local débridement; drinking freely	1.0535	1.054	6.2	6.6 Finger	6.7	0 Opales.	40	39
500 c.c. plasma at 8 A.M.	1.0415	1.041 Finger	4.6	4.6	5.1	0	27	27
	1.0465	1.045 Ear 1.045 Finger 1.046 Toe	4.5	4.4	5.0	0	34	33
	1.0475	1.046	4.4	4.4	5.0	0	36	34
	1.0425	1.040 Ear 1.042 Finger	2.7	2.7	2.9	0	34	31

TABLE III. RELATIONSHIP OF FIBRINOGEN, GRAVITY-CALCULATED FIBRINOGEN, AND THE FORMOL-GEL TEST

PATIENT	FIBRINOGEN: GRAVITY CALCULATED* (GM./100 C.C.)	FIBRINOGEN: TYROSINE (GM./100 C.C.)	FORMOL-GEL: PLASMA	FORMOL-GEL: SERUM	SERUM GLOBULIN (GM./100 C.C.)
1	1.0	0.486	4	0	2.86
2	0.9	0.565	4	0	
3	0.9	0.470	1	0	1.00
				Opales.	
4	0.8	0.285	0	0	
5	0.8	0.532	3	0	
6	0.8	0.476	4	0	
				Opales.	
7	0.8	0.476	1	0	
8	0.8	0.600	4	0	
9	0.8	0.540	4	0	
10	0.8	0.500	4	0	1.40
11	0.7	0.361	0	0	2.50
12	0.7	0.361	0	0	2.11
13	0.7	0.322	3	0	
14	0.7	0.470	0	0	2.73
			Opales.		
15	0.7	0.460	4	0	1.00
16	0.5	0.470	2	0	
17	0.5	0.402	0	0	
			Opales.	Opales.	
18	0.5	0.580	4	0	
19	0.5	0.560	4	0	
20	0.5	0.480	1	0	
21	0.5	0.580	4	0	2.44
22	0.5	0.430	1	0	1.66
23	0.5	0.400	2	0	3.40
				Opales.	
24	0.5	0.290	0	0	1.30
25	0.5	0.302	0	0	1.75
26	0.5	0.340	0	0	1.80
27	0.4	0.386	0	0	
28	0.4	0.340	0	0	
			Opales.		
29	0.4	0.300	0	0	
30	0.3	0.393	4	0	2.20
31	0.2	0.328	0	0	
32	0.2	0.330	1	0	1.25
33	0.2	0.389	0	0	2.10
			Opales.		
34	0.1	0.344	0	0	2.54
35	0.0	0.340	4	0	

*0.0005 was subtracted from the plasma gravity for the anticoagulant.

values, or of inconsistently using the serum value and then the plasma, is evident from the data in Table II. The error is particularly large in those conditions in which fibrinogen production is stimulated;[11] in this series these include burns, extremity wounds with destruction of large muscle masses, penetrating chest wounds with hemorrhage and effusion, and abdominal wounds with peritonitis or hemoperitoneum.

In Table III are data comparing fibrinogen* values with plasma-serum gravity differences. In general, the greater the gravity difference, the higher the fibrinogen value, but no linear relation exists. A closer association exists

*Captain A. Saifer, Sanitary Corps, Seventh Medical Laboratory, performed the fibrinogen and globulin[12, 13] determinations.

between the plasma formol-gel reaction and the fibrinogen level. With four exceptions (Cases 13, 30, 32, and 35) a positive formol-gel is associated with a fibrinogen of 400 mg. per cent or higher. All fibrinogen levels between 400 and 500 mg. per cent are associated with a positive gel of some degree or a definite opacity. Plasma with fibrinogen between 500 and 600 mg. per cent invariably gives a strong reaction.

The serum globulin, when present in normal amount, does not appear to have a significant effect on the formation of the plasma gel. Inasmuch as serum containing more than 4 Gm. per cent globulin forms a gel on the addition of formaldehyde, it is very probable that a certain fraction of the serum globulin, when present in excessive amounts, enters into the formation of the plasma gel. Wise and Gutman,[8] in a study of the serum formol-gel reaction as a test for hyperglobulinemia, warn against the "false positive reactions" occurring in plasma while the corresponding sera are negative. They declare that even moderately increased fibrinogen will cause the plasma reaction to become positive. From our findings it is evident that a close parallelism exists between the amount of fibrinogen and the intensity of the plasma reaction, when the serum globulin is within normal limits.

DISCUSSION

The inability to corroborate Cannon's findings of increased capillary erythrocyte concentration with specific gravity measurements requires examination for the differences in subjects and technique. It is true that shock is treated earlier and more vigorously with plasma and blood in World War II than in World War I. Yet we have studied a sufficient number of patients who had been given no previous infusions, as well as a larger number that were seen in severe shock after having received a minimal amount of plasma and blood. It is unlikely that our consistently negative findings can be due to the more intensive therapy in World War II.

The amount of blood taken from the ear or finger assumes importance if we admit, as Cannon suggests, that in shock the blood corpuscles accumulate in the capillaries of the skin. At best, blood procured by lancet is an admixture of capillary, venous, and arterial blood. The more blood we express, the more it is mixed with venous and arterial blood. Although Cannon does not describe the technique in detail, it may be assumed that the first or second drop was used for the erythrocyte determination. Moreover, a smaller drop is needed for a red cell count than for our gravity measurement. With our method the capillary gravity range could not be accurately anticipated, and from one to three drops of capillary blood were expressed before the correct specific gravity was obtained. The actual effect of this difference in technique could be determined by either using a falling drop method which estimates the rate of fall of a single drop of blood, or by doing simultaneous erythrocyte, hemoglobin, and specific gravity determinations on the capillary blood. Lack of time and facilities prevented us from doing this.

There are several facts which are not easily explained by technical differences. First, Cannon ran hematocrit and hemoglobin determinations and erythrocyte counts in seven cases of shock and found the results to be comparable. Second, in the last stage of our study we determined the venous blood gravity first and then dropped the first drop of capillary blood in this range. Four patients in shock were examined in this manner; one exhibited a capillary gravity 0.004, another 0.003 higher than that of the venous blood (corresponding to a hematocrit 5 volumes per cent and 4 volumes per cent higher, respectively), and the other two showed no significant change. Third, the squeezing associated with filling the capillary capsule caused essentially the same drop in capillary blood specific gravity in those patients in shock as it did in normal subjects.

SUMMARY

1. Simultaneous comparisons of capillary and venous blood gravity in seventy-seven severely wounded soldiers showed no tendency of the capillary blood to be more concentrated than the venous. The gravity difference between capillary and venous blood proved of no value in the diagnosis of the severity of shock.

2. With due attention to the sources of error, capillary blood may be used in place of venous blood for determining hematocrits and serum protein. The effect of shock or edema on capillary values is not greater than the error in the method.

3. As calculated from specific gravities, the proteins of serum are from 0.5 to 1.0 Gm. per 100 c.c. lower than the plasma proteins in those conditions in which fibrinogen production has been stimulated. These conditions include burns, wounds with abundant muscle destruction, chest wounds with hemothorax and effusion, and abdominal wounds with peritonitis and hemoperitoneum.

4. In over 250 determinations on wounded soldiers studied up to ten days after injury, the serum formol-gel reaction was faintly positive on only three occasions. There is a close correlation between the fibrinogen level and the plasma formol-gel reaction. The latter does not appear to be affected by serum globulin that is within the normal range.

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STUDIES IN COMPLEMENT FIXATION

II. Preservation of Sheep's Blood in Citrate Dextrose Mixtures (Modified Alsever's Solution) for Use in the Complement Fixation Reaction

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With the Technical Assistance of Rebecca Goodman, M.T.

THE increasing use of preserved human blood for transfusion purposes has stimulated investigations designed to establish ideal blood-preserving solutions.[1-8] The best of these have employed varying isosmotic concentrations of dextrose, citrate, and sodium chloride at slightly differing hydrogen-ion concentrations. The blood is stored in the various solutions in final concentrations ranging from 20 to 50 per cent, the dilution factor itself apparently influencing the efficiency of preservation. Kendrick and associates[9] recently found modified Alsever's solution* as acceptable as any tested and, employing this solution, developed a practical method of supplying preserved blood to the European Theater of Operations.

A preservation technique for maintaining uniformity in the properties of sheep's erythrocytes would be a great laboratory convenience. Most laboratories at present prepare suspensions of cells from defibrinated blood or blood collected into sodium citrate solution with or without the formalin advocated by Kolmer[10]; cells so preserved have been stated to remain satisfactory for use over short periods. It would be of great advantage to employ preserving fluids which would maintain the properties of sheep's blood over long periods and during practical methods of transportation. Quantitative studies in this laboratory indicate that the aseptic collection of sheep's blood in modified Alsever's solution at ordinary temperatures and subsequent refrigeration permits the preservation of the blood for at least ten weeks (the duration of the experiment) without development of appreciable hemolysis or change in susceptibility to lysis by guinea pig complement and rabbit amboceptor.

EXPERIMENTAL

Glass vaccine vials, calibrated at 30 ml., were fitted with rubber stoppers and evacuated. Using aseptic precautions, a series of eight vials were prepared to contain, respectively, 6, 9, 12, 15, 18, 21, 24, and 27 ml. of sterile modified Alsever's solution. Five milliliters of sterile 4 per cent sodium citrate were added to a ninth vial. Each vial was then aseptically filled to the 30 ml. mark with sheep's blood obtained at a single bleeding with the aid of a donor set of

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*Each 100 ml. of Alsever's solution contains: dextrose, 2.05 Gm.; sodium citrate, 0.8 Gm.; and sodium chloride, 0.42 Gm. The original Alsever formula has been recently modified by the addition of 0.055 Gm. of citric acid per 100 ml. The finally sterilized solution has a pH of 6.1 and shows very slight or no evidence of caramelization.

the type described by Elliott and Nessett.[11] The resulting suspensions, varying in blood concentration from 10 to 80 per cent, were stored at from 3 to 6° C. for the duration of the experiment and were mixed only before each removal of samples for analysis. At intervals of two, six, ten, seventeen, thirty-four, fifty-three, and seventy-four days after bleeding, the blood suspensions were mixed thoroughly by inversion and appropriate quantities removed aseptically. Portions containing 0.25 ml. of each mixed sample were added to a quantity of distilled water calculated to yield a 1:125 final dilution of the whole blood content of the sample, and the optical density of the resulting lysate was determined on the Coleman Junior Spectrophotometer* at 580 mμ. A portion of the supernatant fluid of the sample, obtained after centrifugation, was similarly diluted and the optical density determined. The optical density of the diluted supernatant divided by the optical density of the diluted whole blood lysate × 100 was taken as the percentage of spontaneous hemolysis which had occurred during storage. Samples of each mixture were then washed with four changes of M/200 phosphate buffered saline, pH 7.3, and the volume of packed cells read in calibrated centrifuge tubes after a final centrifugation at 2,000 r.p.m. for ten minutes. The packed cells from each sample were brought to 2 per cent concentration by diluting to 50 volumes with buffered saline, and the optical density of a lysed 1:5 dilution of each 2 per cent suspension in distilled water was determined at 580 mμ.

A portion of the 2 per cent suspension made from the 50 per cent blood mixture was sensitized with an equal volume of the optimal rabbit amboceptor dilution,[12] and the quantity of guinea pig complement required for 50 per cent hemolysis was determined by spectrophotometric titration.[13] Then portions of the other 2 per cent suspensions were sensitized similarly with rabbit amboceptor, and the percentage of hemolysis obtained with the above 50 per cent unit of complement was determined for each suspension. The 50 per cent unit for each suspension then was calculated with the aid of predetermined volume factors,[13] and the remainder of the 2 per cent suspensions was stored overnight at from 3 to 6° C. On the following day portions of the stored 2 per cent suspensions were sensitized, and the 50 per cent hemolytic units again were determined in the same fashion using the lot of complement employed for the previous day's tests. Finally, a portion of each stored 2 per cent suspension was centrifuged at 2,000 r.p.m. for ten minutes, and the optical density of the undiluted supernatant fluid was determined at 580 mμ. The percentage of cells hemolyzed during overnight storage of the 2 per cent suspension was determined from the following formula:

$$\text{Percentage of hemolysis} = \frac{\text{Optical density of undiluted supernatant}}{\text{Optical density of 2 per cent cells} \times 5} \times 100$$

RESULTS

The 80 per cent blood mixture, in which the final concentration of citrate was only 0.16 per cent, had clotted completely after six days, and the 70 per

*Model 6, manufactured by Coleman Electric Co., Maywood, Ill., and New York, N. Y.

TABLE I. QUANTITATIVE STUDIES WITH PRESERVED SHEEP'S BLOOD

I BLOOD MIXTURE	II DAYS AFTER BLEEDING	III SAMPLE (ML.)	IV PACKED CELLS (ML.)	V O.D.* OF MIXTURE 1:125	SUSPENSION VI O.D. OF HEMOLYZED 2% CELLS	SUSPENSION VII 50% UNIT† (ML. × 10^{-3})	RESULTS WITH 2% SUSPENSION AFTER STORAGE OVERNIGHT VIII 50% UNIT† (ML. × 10^{-3})	RESULTS WITH 2% SUSPENSION AFTER STORAGE OVERNIGHT IX HEMOLYSIS DURING STORAGE (PER CENT)
IV	2	3.0	0.62	.490	.514	1.93	2.0	0
Alsever's (15 ml.)	6	3.0	0.62	.458	.523	1.80	1.87	0
Blood (15 ml.)	10	3.0	0.70	.470	.485	1.63	1.59	0
	17	3.0	0.68	.450	.510	1.81	1.55	0
	34	3.0	0.55	.472	.512	1.78	1.86	0
	53	3.0	0.66	.452	.495	1.63	1.57	1.1
	74	3.0‡	0.62	.490	.474	1.54	1.61	0.8
IX	2	2.0	0.70	.462	.497	1.95	2.08	0.0
4 per cent citrate	6	2.0	0.75	.452	.483	1.78	1.80	0.6
(5 ml.)	10	2.0	0.84	.450	.479	1.64	1.64	0.8
Blood (25 ml.)	17	2.0	0.80	.472	.503	1.80	1.58	0.8
	34	2.0	0.80	.470	.497	1.79	1.77	0.8
	53	2.0	0.75	.460	.498	1.62	1.65¶	5.1
	74	2.0§	0.70	.452	.456	1.55	1.69¶	11.7

*O.D., Optical dentistry determined at 580 mμ.

†Complement unit.

‡1.8 per cent hemolysis in this sample.

§2.5 per cent hemolysis in this sample.

¶These represent the unit of complement required for the hemolysis of residual intact cells. In the course of the titration the color due to spontaneous hemolysis of the 2 per cent suspension was eliminated by the use of a blank prepared from its supernatant fluid.

cent blood mixture showed a few small clots after several weeks. Clotting did not occur in the remaining mixtures. In Table I are summarized the data obtained with the 50 per cent blood-Alsever's mixture (Suspension IV) and the parallel data obtained with the citrated blood mixture (Suspension IX). Results obtained with the 20, 30, 40, and 60 per cent blood mixtures were as satisfactory as those recorded for the 50 per cent mixture. The yields of packed cells obtained and the optical densities of the original blood mixtures and of the 2 per cent suspensions prepared therefrom were quite constant, suggesting that there was no tendency toward irreversible change in cell size during the course of storage. Spontaneous hemolysis of 2.5 per cent was observed in the simple citrate blood mixture after seventy-four days and was only slightly greater than that observed with the 50 per cent Alsever's mixture. Daily variations in the absolute quantity of complement required for 50 per cent hemolysis of the 2 per cent suspensions from the given mixture are the expected variations obtained when different ampules of sublimated complement are employed. The 50 per cent unit required for Suspension IV was also in very close agreement with that required for Suspension IX. It is especially noteworthy that the 2 per cent suspensions after storage overnight remained quantitatively unchanged in their response to lysis by complement and amboceptor. This was equally true of the *unhemolyzed* cells of the citrate blood mixtures at fifty-three and seventy-four days. The use of these 2 per cent suspensions in complement fixation tests employing an overnight fixation period, however, is contraindicated by the occurrence of 5 and 11 per cent hemolysis in the stored 2 per cent suspensions.

Such a degree of hemolysis leads to errors in estimating the degree of inhibition of hemolysis by serum plus antigen in an overnight fixation test. Since many complement fixation procedures are most sensitive after eighteen hours of refrigeration of test mixtures, this diminishes the usefulness of cells preserved in citrate alone.

This laboratory has tentatively adopted the use of modified Alsever's solution for the preservation of sheep corpuscles while additional studies are in progress. One hundred fifty milliliters of sheep's blood is collected aseptically in a sterile vacuum bottle containing 150 ml. of modified Alsever's solution. After thorough mixing, the mixture is aseptically dispensed into six sterile 50 ml. evacuated vaccine vials and stored at refrigerator temperature. Quantities of blood mixture are aseptically withdrawn from these vials as needed. Maintenance of strict asepsis is facilitated by introducing a sterile cotton-filled airway into the rubber cap before withdrawing the desired quantity of the blood mixture into a sterile syringe. In our experience with several sheep, 15.0 ml. of such a mixture will yield sufficient packed cells for 200 ml. of a 2 per cent suspension. On one occasion, a vial of preserved blood was flown to the Caribbean area where it remained at room temperature for two weeks and was then flown back to this laboratory. Upon arrival here, the mixture showed no hemolysis, and a 2 per cent suspension prepared from it required the same complement unit for 50 per cent hemolysis as a 2 per cent suspension prepared at the same time from a vial of the same mixture which had been refrigerated at this laboratory throughout this period. After three days in the refrigerator, however, the blood mixture which had been at room temperature for several weeks underwent considerable hemolysis. This was apparently a delayed osmotic hemolysis of the type encountered with human blood by DeGowin and associates[5] in the use of a modified Rous-Turner preserving mixture. Under ordinary circumstances, however, sheep's blood collected in Alsever's solution at room or mild outdoor temperatures can be subsequently refrigerated without undergoing osmotic hemolysis. This is similar to the experience of Kendrick and co-workers[9] in the use of modified Alsever's solution for the collection of human blood. The uniform behavior of cells preserved in this fashion is further attested by the fact that the slope of the curve of hemolysis with complement remains constant throughout the period of storage.[13]

Several blood-citrate mixtures were also prepared according to the method of Ashby.[14] Since considerable hemolysis occurred fairly rapidly, both in these stored mixtures and in washed 2 per cent suspensions prepared from them, the recommended citrate mixture was considered inferior to Alsever's solution. Twenty-five milliliters of sheep's blood were also treated with 6 Gm. of Amberlite, as recommended by Steinberg.[15] After only seven days of preservation, considerable hemolysis occurred following refrigeration overnight of 2 per cent suspensions prepared from this mixture. After eleven days of preservation there was rapid hemolysis of this blood, and packed washed cells were resuspended with difficulty. It should be noted, however, that the original cells and the residual unhemolyzed cells of the later mixtures showed quantitatively the

same susceptibility to lysis by complement as did cells preserved in citrate or citrate-dextrose mixtures.

Preliminary studies have also been made with a citric acid-sodium citrate-glucose mixture prepared as recommended by Loutit and associates.[2] Moderate clot formation occurred shortly after the mixture was made, probably due, as Loutit and associates suggest, to the relatively low concentration of citrate radical which required that greater care be taken with this solution to assure thorough mixing. In addition, 19 per cent hemolysis had occurred in the mixture after only forty-eight days of storage, an amount considerably greater than that encountered with Alsever's solution.

No attempt was made to assess the comparative value of 90 per cent blood mixtures containing final concentrations of 0.5 per cent sodium citrate and 2 per cent dextrose as recently recommended by Coffin.[16] It should be noted that Coffin encountered considerable hemolysis in his mixture exposed to 85° F., which constitutes a disadvantage in its use. It is possible that these mixtures undergo isosmotic hemolysis of the type described by DeGowin and co-workers.[5]

DISCUSSION

The duration of preservation of sheep's blood for use in the complement fixation reaction is dependent upon the same factors which influence the preservation of human blood; these factors are: (1) strict asepsis during the withdrawal, storage, and daily removal of the blood sample, (2) adequacy of the preserving fluid and the use of optimal blood-preserving fluid ratios, and (3) storage under refrigeration.

The necessity of maintaining absolute asepsis in all stages of handling the sheep's blood cannot be overemphasized. Contamination of the stored mixture will ordinarily result in prompt and progressive hemolysis of the cells. The use of commercially available* sterilized and evacuated bottles containing the required amount of modified Alsever's solution greatly facilitates maintenance of absolute asepsis. An evacuated 300 ml. bottle containing 150 ml. of Alsever's solution provides a most convenient unit for the collection of 150 ml. of blood. Under these conditions it is not necessary to use a bleeding set equipped with a valve; a short length of rubber tubing equipped with a small screw clamp and fitted with a 16-gauge needle at one end and 17-gauge at the other will serve as a most satisfactory bleeding unit.

The present investigation has not attempted an exhaustive survey of preserving fluids for use in prolonged storage of sheep's blood. Modified Alsever's solution, used with an equal volume of blood as described, proved sufficiently satisfactory to meet the needs of the average serologic laboratory, and it has been adopted for that reason. Later studies may well define other preserving fluids capable of extending the time of storage of sheep's blood well beyond that described in the present instance.

It is of considerable interest that blood preserved in Alsever's solution will survive storage at room temperature for appreciable periods. This property

*Baxter Centri-Vac, manufactured by Baxter Laboratories, Inc., Glenview, Ill., and College Point, N. Y.

makes it possible to transport such blood mixtures for considerable distances without necessitating refrigeration during shipment. The limitations of room temperature shipment as a practical method of dispatching sheep's blood from a central distributing point are still under investigation.

SUMMARY

Sheep's blood, aseptically collected in equal volumes of modified Alsever's solution at ordinary temperatures, may be preserved under refrigeration for at least ten weeks without significant alteration in susceptibility to lysis by complement and amboceptor. Blood collected in this fashion has been transported and maintained at ordinary temperatures for at least two weeks with no change in these properties.

The preservation of sheep's blood in modified Alsever's solution as described may not yet be the best available method. It has been exclusively used in this laboratory, however, during the past ten months with complete satisfaction.

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HOMOLOGOUS AND HETEROLOGOUS STRAINS OF PLASMODIUM VIVAX: A CROSS-INOCULATION STUDY OF MALARIA STRAIN IMMUNITY

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PREVIOUS investigations of the immune response to vivax malaria have demonstrated that strains of *Plasmodium vivax* differing in geographic origin are dissimilar in their stimulation of the immune mechanism.[1] This difference between substrains of the same species of malaria is best illustrated by the technique of cross-inoculation, whereby a heterologous strain produces definite clinical activity following the reinoculation of a patient who had previously experienced clinical activity with another strain. Homologous strains are represented by minimal or absent clinical activity on reinoculation in the same individual. The demonstration of these characteristics of homologous and heterologous strain immunity on a quantitative basis has aided in solving the provocative problem of dealing with the partially immune response to malaria inoculations in the treatment of neurosyphilis.

Variations in the susceptibility of different individuals to therapeutic malaria have been adequately considered in a previous publication.[2] Because of apparent native immunity to vivax malaria, all Negroes, Puerto Ricans, Mexicans, and Mediterranean peoples are primarily inoculated with quartan malaria at this institution. Similarly, all white patients with definite histories of previous attacks of natural malaria are initially inoculated with a quartan strain. The real problem, therefore, arose in the group of other white individuals, without definite histories of malaria, who experienced partially immune responses following vivax inoculation.

Partial immunity is recognized by the characteristics of both the clinical course and parasitemia of the inoculated individual. In these cases, parasites usually increase in density to several hundred per cubic millimeter before there is any febrile activity. Usually the parasite count drops spontaneously and precipitously after from three to seven paroxysms, and the patient becomes completely afebrile. Partially immune patients are divided into two groups: (A) Those experiencing less than five paroxysms of fever to 103° F. or more and (B) those experiencing from five to eight such episodes. This segregation was made to conserve time in the completion of a therapeutic course. At first it seemed logical, even though a previous history of natural malaria was not elicited from the patient, to reinoculate all partially immune patients with quartan malaria

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for the completion of their therapy. Two objections to this procedure were evident. First, reinoculation with quartan malaria, particularly in an individual who has already showed a certain degree of malaria immunity, was followed by a prolonged incubation and prepatent period, often lasting four or more weeks. This, plus the longer clinical course of quartan malaria (paroxysms every third day), substantially increased the hospitalization and morbidity of the patient. Second, the prolonged latency of quartan infections, reportedly thirty years or more in some instances, may actually play a role in the production of accidental transfusion malaria. In a recent report[3] of twelve patients with transfusion malaria in Massachusetts, nine of the twelve were quartan cases, two were tertian (vivax), and the etiologic species of one was not stated. It is, of course, recognized that these latent quartan infections were probably produced by the mosquito inoculation of the natural disease; and, although it is presently believed that blood inoculation is not followed by such latency, the procedure must be considered somewhat hazardous in this respect until further information is available. For these reasons, heterologous strains of vivax malaria were first utilized for the reinfection of those white patients demonstrating partially immune responses (Group B) to the original vivax inoculation. Group A patients were routinely reinoculated with quartan malaria because of their relatively high degree of vivax immunity. If adequate clinical activity (a total of eight to twelve paroxysms) can be produced by the use of heterologous strains, the prolonged reinoculation incubation period would be eliminated and the problem of prolonged latency obviated by the shorter vivax infection. It was the purpose of this study, therefore, to demonstrate the characteristics of homologous and heterologous strain immunity by a controlled cross-inoculation investigation. An additional refinement in the techniques of therapeutic malaria may thus become available.

The principle of reinoculation of previously vivax-infected individuals with heterologous strains of *P. vivax* is not a new one. Boyd,[1] in a description of the criteria of immunity and susceptibility in naturally induced vivax malaria infections, in 1942, demonstrated the difference between homologous and heterologous vivax immunity with different strains endemic to the United States. Of a series of twenty-five patients reinoculated with a known homologous strain of *P. vivax,* seventeen experienced subclinical "takes" and eight short clinical attacks. On the other hand, reinoculation in a series of nineteen patients with heterologous strains of *P. vivax* produced subclinical infection only once and clinical activity of varying duration in the remaining eighteen patients. The primary inoculation strain in that study was *P. vivax* McCoy, indigenous to Southeastern United States. The heterologous strains were obtained from other sections of the country.

The treatment at this installation of soldiers from overseas theaters facilitated the recovery of strains of *P. vivax* from patients originally infected in several different Southwest Pacific islands. These so-called Southwest Pacific strains, because of their more distant geographic origin, were utilized in this cross-inoculation study against the *P. vivax* McCoy strain. The results may be

of increasing value in the management of neurosyphilis with therapeutic malaria in a population manifesting greater malaria immunity as a result of the prolonged residence of soldiers in hyperendemic areas.

MATERIAL AND METHOD

Origin of Strains.—Five strains of *P. vivax* from different geographic regions were used in this study.

Strain M: The McCoy strain, carried through successive mosquito-human transfers for the past fifteen years at the Florida State Hospital, Chattahoochee, Fla. This strain was originally recovered from a patient infected in the Florida-Alabama area and is the one currently used for the primary inoculation of susceptible white patients.

Strain A: The DuBose strain, recovered during the eighth malaria recurrence from a patient who first contracted the disease on Guadalcanal Island in November, 1942. He had had no previous experience with malaria prior to that attack and had been on no other Southwest Pacific island.

Strain B: The Jenkins strain, recovered during the second malaria recurrence from a patient first infected in February, 1944, on New Guinea Island.

Strain C: The Fort strain, recovered from a patient who had been on both New Guinea and Bougainville Islands, with the first attack of malaria occurring in the latter area.

Strain D: The Siimes strain, recovered during the fourth malaria recurrence from a patient whose first clinical attack occurred in December, 1942, on New Guinea Island.

Technique of Cross-Inoculation.—Of a total of 265 patients with neurosyphilis treated with therapeutic vivax malaria during the past year, thirty-five white patients were included in this study. Of these thirty-five patients, only twenty-seven were included in the final analysis of results. Of the eight patients not completely analyzed, three were originally inoculated intradermally and demonstrated negative parasitemias for four weeks following inoculation. On subsequent intravenous inoculation with the same strain of vivax malaria, these patients ran courses typical of susceptible individuals. Two other patients were inoculated with infected mosquitoes (a minimum of two infected mosquitoes positive on dissection), but they also experienced negative parasitemias for four weeks postinoculation. The confusing results in these five patients receiving intradermal and mosquito inoculations were attributed to technical difficulties. The remaining three patients excluded from the study were strongly suspected of illegally consuming atabrine during their hospitalization.

The original vivax inoculation in most instances was performed intravenously, but a few successful mosquito and intradermal inoculations were also employed. Clinical activity following the original inoculation persisted until the occurrence of a spontaneous remission. No therapeutically interrupted original infections were included in this series because of the greater possibility of active recurrence of the original strain later in the patient's hospitalization. In spite of the negative histories of previous malaria attacks in twelve patients,

most of them had some degree of malaria immunity at the onset, with spontaneous remissions occurring prior to the exhaustion of the patient by a prolonged number of paroxysms. Reinoculation and further clinical activity was thus feasible within the therapeutic realm.

Following each spontaneous remission after the original inoculation, interval quinine therapy (10 gr. per 50 pounds of body weight daily for five days) was withheld until the parasitemia decreased to submicroscopic densities. This was merely an added precaution in sterilizing the blood stream prior to reinoculation. From three to five days following the completion of quinine therapy, an adequate interval for the excretion of the drug, reinoculations with either homologous or heterologous vivax strains were accomplished. All reinoculations were performed intravenously, but the total quantitative parasite dosage was varied in order to estimate the effect of the parasite dosage on the prepatent and incubation periods in patients whose immunity was heightened by recent malaria attacks. Following this reinoculation, clinical activity ensued until the development of a second spontaneous remission. Routine atabrine therapy to terminate completely the infection was withheld until the second parasitemia was either negative or at insignificant levels at the end of two weeks following the last bout of clinical activity.

An example of the cross-inoculation technique in an individual case can be summarized from Table I. Patient 153 was originally infected with strain M (*P. vivax* McCoy) and experienced fourteen paroxysms reaching 103° F. or more and twenty-seven days of clinical activity. Following a spontaneous remission, this patient received five days of interval quinine therapy. Three days later, he was reinoculated with strain A (*P. vivax* DuBose). He then experienced five additional paroxysms reaching 103° F. or more and sixteen days of clinical activity. After a second spontaneous remission, routine atabrine therapy was finally instituted.

The Reinfection Index.—Since this study was primarily concerned with the relationship of different vivax strains to each other, it was necessary to fix some factor which would largely cancel the effect of the varied individual malaria immunity prior to original inoculations. For example, if on original inoculation a patient experiences eighteen paroxysms, he is much more susceptible to the disease than the patient who experiences only four paroxysms initially. In the event, however, that each of these patients, on subsequent reinoculation, experiences two paroxysms, it cannot be assumed that the relationship of the reinfection strain to the original strain was the same in both cases, since one's immunity to malaria was much greater than the other's prior to the experiment. A simple method of cancelling this pre-existing individual immunity as a factor in determining strain difference was devised. The number of paroxysms experienced after reinfection was calculated as a percentage of the number of paroxysms experienced after original infection. This percentage figure was designated the *reinfection index.* To follow the previous example, the reinfection index, in the case of the patient experiencing eighteen fevers on original infection

TABLE I.—CROSS-INOCULATION DATA

PATIENT	HOME STATE	STRAIN ORIG.	STRAIN REIN.	HISTORY OF MALARIA	PAROXYSMS ORIG.	PAROXYSMS REIN.	PAROXYSMS R.I.	DAYS OF CLINICAL ACTIVITY ORIG.	DAYS OF CLINICAL ACTIVITY REIN.	DAYS OF CLINICAL ACTIVITY R.I.
347	Ga.	M	M	Malaria, 1935	18	2	11.1	21	4	19.0
153	Ind.	M	A	Negative	14	5	35.7	27	16	59.3
157	Ala.	M	B	Malaria, 1938 (Ala.)	6	4	66.7	8	7	87.5
455	Ga.	M	C	Malaria, 1930 (Fla.)	7	2	28.6	9	3	33.3
349	N. Y.	M	D	Native-born Greek	4	2	50.0	9	3	33.3
442	Ga.	A	M	? Malaria (Panama)	4	10	250.0	9	22	244.0
514	Ky.	A	A	"Chills," 1930 (Ky.)	4	0	0.0	5	0	0.0
398	Va.	A	B	Negative	15	4	26.7	17	7	41.2
501	S. C.	A	C	? Malaria as child	3	1	33.3	4	3	75.0
418	Ala.	A	D	Negative	8	3	37.5	12	6	50.0
345	S. C.	B	M	Negative	12	4	33.3	22	7	31.8
239	Fla.	B	A	Malaria, 1937 (Fla.)	9	5	55.5	11	5	45.5
384	S. C.	B	B	Negative (but in Southwest Pacific)	12	1	8.3	23	3	13.0
239	Fla.	B	C	Malaria, 1937 (Fla.)	9	8	89.0	11	9	81.8
326	Va.	B	D	Negative	10	8	80.0	16	13	81.3
400	Ohio	C	M	Malaria, ? 1941	13	7	53.8	26	10	38.4
239	Fla.	C	A	Malaria, 1937 (Fla.)	8	5	62.5	9	5	55.5
514	Ky.	C	A	"Chills," 1930 (Ky.)	4	3	75.0	8	5	62.5
261	Texas	C	B	Negative	6	3	50.0	14	7	50.0
424	Ga.	C	C	Malaria, 1935 (Ga.)	11	0	0.0	13	2	15.4
218	Okla.	C	D	Negative	12	3	25.0	24	7	29.2
539	Ala.	C	D	Malaria, 1939 (Ky.)	7	10	143.0	10	20	200.0
397	La.	D	M	? (Paretic)	6	4	66.7	8	7	87.5
294	Texas	D	A	Mexican	3	4	133.3	3	7	233.3
263	Texas	D	B	Negative	1	1	100.0	1	1	100.0
296	N. Y.	D	C	Negative	5	7	140.0	8	9	112.2
397	La.	D	D	? (Paretic)	6	2	33.3	8	3	37.5

Strains of *P. vivax:* M, McCoy; A, DuBose; B, Jenkins; C, Fort; D, Siimes.

Orig., Original infection; Rein., reinfection; R.I., reinfection index (per cent). The figures for paroxysms following inoculation apply to those reaching 103° or more; the days of clinical activity include the time interval from the onset of the first febrile elevation to the cessation of clinical activity for that infection; the number of the patient represents the center number of each patient.

and two on reinfection, would be 11.1. On the other hand, the index would be 50.0 in the case of the patient developing four fevers on original infection and two on reinfection. The higher the reinfection index, therefore, the greater is the difference between the strains cross-inoculated.

Although other studies of malaria immunity base the duration of an individual infection on the days of clinical activity from the onset of elevation in temperature until its permanent cessation, this estimation cannot readily be evaluated in terms of therapeutic adequacy in the treatment of neurosyphilis. The usual criterion of adequate malaria therapy is the actual number of paroxysms reaching a certain febrile height (103° F. or more) rather than the duration of total clinical activity which includes fever-free days. Since this study was undertaken for the purpose of applying the results to therapeutic malaria, the reinfection indices, based on number of paroxysms only, were analyzed. A comparison of the indices by both methods, however, reveals little significant difference (see Table I).

RESULTS

The clinical implications of this cross-inoculation study can be evaluated only after a determination of the immunity characteristics of the series, with a reduction of the patients' varied previous experience with malaria or residence

in endemic areas to a common level. It is our belief that the reinfection index is the single implement most effectively used in judging the results on a common basis. It may be of interest, however, to analyze the series even more closely. Eighteen of the twenty-seven patients (66.6 per cent) experienced less than ten paroxysms on original infection, indicating partial immunity of some type. Only fourteen of the twenty-seven patients had definite previous histories of attacks of natural malaria, but three of those had more than ten paroxysms following original inoculation and apparently had never gained any great degree of immunity from their natural infection. Since Negro patients are highly immune to vivax malaria, only white patients were considered in this study; one, however, was Mexican. Of the thirteen patients with no histories of previous infection, one, whose story was unreliable, had had early paresis.

An analysis of the home states of patients listed in Table I reveals that of the four patients with negative histories who developed partially immune clinical courses, two were from Texas, one was from New York, and one was from Alabama. Three, therefore, may have resided for long intervals in endemic areas. One other consideration was important in evaluating the results. If a patient had experienced a previous attack of natural malaria in one of the Southwest Pacific islands from which our strains were recovered, he might have an adequate infection when inoculated originally with Strain M (Southeast United States), but he might then show practically no clinical activity when reinfected with one of the other strains. In such a case, it would be impossible to distinguish between homologous immunity to the original induced infection or homologous immunity to the original natural attack in the endemic region from which our reinfection strain originated. Confusing as this appeared to make matters, it did not enter extensively into the interpretations, for only one patient had experienced his natural attack outside continental United States, and that was in Panama, not the Southwest Pacific islands.

HOMOLOGOUS IMMUNITY

The mean value (Table II) for the duration of paroxysms following original infection in the five patients reinfected with homologous strains was 10.2. The duration of paroxysms in this group on reinfection averaged 1.0. These figures alone denote the characteristics of homologous immunity, with reinfection with

TABLE II. SUMMARY OF MEAN VALUES

Patients reinfected with homologous strains (five)	
Duration of paroxysms following original infection	10.2
Duration of paroxysms following reinfection	1.0
Reinfection index	10.5
Patients reinfected with heterologous strains (twenty-two)	
Duration of paroxysms following original infection	7.5
Duration of paroxysms following reinfection	4.5
Reinfection index	74.3
Individual heterologous reinfection indices of each strain	
M vs. A, B, C, D	73.1
A vs. M, B, C, D	78.8
B vs. M, A, C, D	62.6
C vs. M, A, B, D	70.0
D vs. M, A, B, C	86.1

similar strains producing practically no further clinical activity. The homologous reinfection index, mean value, was 10.5. Practically applied, this would mean that rarely would one expect to induce a total reinfection with homologous strains which would last for more than one-tenth of the duration of the original infection. The use of homologous strains in completing therapeutic vivax malaria in patients demonstrating partial immunity is, therefore, not practicable.

HETEROLOGOUS IMMUNITY

The mean value (Table II) for the duration of paroxysms following original infection in the group of twenty-two patients reinfected with heterologous strains was 7.5. However, the duration of paroxysms following reinfection with these heterologous strains was 4.5. Thus, in spite of the fact that the group as a whole displayed a greater degree of pre-existing malaria immunity, they developed more prolonged reinfection clinical activity than did the group reinfected with homologous strains. The heterologous reinfection index (mean value, 74.3) clarifies this differentiation. The reinfection of a previously vivax-infected patient with a heterologous strain would, on the average, produce clinical paroxysms totaling approximately 75 per cent of the clinical paroxysms experienced on original infection. Further analysis reveals that fifteen of the twenty-two (68.2 per cent) reinfected with heterologous strains showed secondary clinical activity greater than 50 per cent of the primary course. Four of the twenty-two (18.2 per cent) showed from 31 to 40 per cent reactivity and three of the twenty-two (13.6 per cent) between 25 and 30 per cent. If heterologous strains of vivax malaria were, therefore, used to reinfect patients experiencing partial immunity (Group B, from 5 to 8 paroxysms) following original infection, 68.2 per cent would experience therapeutic courses totaling more than 50 per cent of their original bouts and 86.4 per cent more than 31 per cent of their original courses.

Table II lists the mean values of the reinfection indices when heterologous reinfection was employed against each single strain in turn. The figures varied from 62.6 to 86.1. It cannot be said with certainty, therefore, that any one of the strains was very much more active than any other in heterologous reinfection. Clinical observations, however, indicated that strain A (DuBose) and strain D (Siimes) were the most potent in producing severe infections with longer, more intense paroxysms. Extensive experience with Southwest Pacific strains during the past year has disputed the common impression that they are more virulent than the United States strains utilized as therapeutic agents. Complications encountered during therapeutic malaria with these two types of *P. vivax* have been strictly comparable.

The relationship of the reinoculation intravenous parasite dosage to the duration of the prepatent and incubation periods of the reinfection can be studied from the tabulation of mean and median values listed in Table III. Included as a comparison against the twenty-one heterologous cross-inoculation cases is a control series of seventy-eight patients, all of whom experienced completely susceptible courses of vivax malaria. It is first noted that, in partially

TABLE III. RELATION OF INOCULATION PARASITE DOSAGE TO PREPATENT AND INCUBATION PERIODS

PARASITE DOSAGE	CONTROL GROUP					CROSS-INOCULATION GROUP				
	NUMBER OF PATIENTS	P.P. PER		INC. PER		NUMBER OF PATIENTS	P.P. PER		INC. PER	
		ME.	MD.	ME.	MD.		ME.	MD.	ME.	MD.
0 to 10 mil.	35	6.8	6.0	7.6	7.0	7	3.7	3.0	6.6	6.0
11 to 30 mil.	20	3.3	3.0	3.8	3.0	7	6.4	7.0	11.9	12.0
31 to 150 mil.	23	2.9	3.0	2.5	2.0	7	3.1	3.0	8.0	9.0

P.P. Per., Prepatent period; Inc. Per., incubation period; Me., mean (average); Md., median; mil., millions.

immune individuals, the incubation period is invariably longer than the prepatent period, the difference varying from three to six days in this series. This confirms the fact that clinical activity in a partially immune patient begins only after the parasite density attains high levels and rarely when the density is submicroscopic. This is in contrast to the control series of susceptible patients, where the prepatent and incubation periods are of almost equal duration. Further examination reveals that in susceptible patients, the prepatent and incubation periods vary inversely with the inoculation parasite dosage, with the longest periods following the smallest inocula and the shortest periods the largest inocula. This relationship, however, is not maintained in any group of patients with varying degrees of immunity, since such immunity predetermines to some extent the duration of these periods. This is indicated statistically in Table III (cross-inoculation group), in which it is noted that longer pre-patent and incubation periods occur with higher parasite dosages (from 11 to 30 millions) than with lower dosages (from 0 to 10 millions).

SUMMARY

The problem of partially immune responses to vivax inoculation in white patients with neurosyphilis who have no previous history of attacks of natural malaria has been a provoking one. These patients, part of an ever-increasing group occasioned by the prolonged residence of soldiers in hyperendemic overseas areas, cannot readily be screened out for inoculation with other species of malaria because of their negative histories. They may, therefore, require reinoculation for the completion of therapeutic courses.

The present custom of reinoculating these patients with quartan malaria raises two objections: (1) Quartan reinoculation materially prolongs hospitalization and morbidity because of the prolonged incubation period and the longer duration and cycle of paroxysms and (2) quartan infections, differing from the other species of malaria, often become latent for many years and may recur or act as the source of accidental transfusion malaria.

Homologous immunity is characterized by the production of minimal clinical activity in patients reinfected with the same or similar substrain of vivax malaria. The duration of the homologous reinfection in this series of patients averaged 10.5 per cent (reinfection index) of the duration of the original infection with that same strain.

Heterologous immunity exists when reinfection of a patient with a strain of *P. vivax* differing in geographic origin from the original strain results in definite clinical activity sufficient for the completion of a course of malaria therapy. In this cross-inoculation study, the duration of the heterologous reinfection averaged 74.3 per cent (reinfection index) of the duration of the original infection.

In a control series of seventy-eight susceptible patients, intravenous inoculation with various parasite dosages of *P. vivax* revealed that the subsequent prepatent and incubation periods varied inversely with the total quantitative dosage. This correlation, however, does not apply to any series of patients with immunity to malaria.

It seems desirable, as a result of malaria immunity studies, to reinoculate with heterologous strains* of *P. vivax* those white patients with neurosyphilis experiencing partially immune types of original infection (from five to eight paroxysms). Adequate completion of therapeutic courses and avoidance of unnecessary quartan infections thus can be accomplished.

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*Heterologous Southwest Pacific strains of *P. vivax* are currently available at the Florida State Hospital, Chattahoochee, Fla.

BACTERIOLOGIC STUDIES OF THE SPUTUM IN PATIENTS WITH PNEUMOCOCCAL PNEUMONIA TREATED WITH PENICILLIN

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A STUDY of the sputum of patients with pneumonia often provides a means of following the bacteriologic response to various forms of therapy and gives some insight into the mechanism of action of the therapeutic agents. Changes in the sputum may reflect to a considerable degree those which occur in the lung. Such studies have previously been made, chiefly by Frisch,[1-12] in some patients receiving only supportive therapy and in others who were treated with specific antisera or sulfonamide drugs.

The result of various forms of effective therapy is a reduction in the number of organisms in the sputum and in the lungs. In untreated patients, agglutination of pneumococci in the sputum is sometimes, though not always, observed between the sixth and tenth day of the disease. Adequate therapy with type-specific antipneumococcus serum brings about the agglutination of pneumococci in the sputum within from six to twelve hours. This is usually followed by phagocytosis and by a gradual reduction in the number of organisms over a period of several days. The sulfonamides exert a direct bacteriostatic effect which usually results in a prompt reduction in the number of pneumococci within from twelve to thirty-six hours. There is, however, considerable variation in the persistence of pneumococci in sulfonamide-treated patients, and they persist for long periods in about 30 per cent or more of patients so treated.[13, 14] The persistence of pneumococci during the sulfonamide therapy is not related to the failure of the patient to develop homologous antibodies.[14] It may be associated with the development of resistance of the pneumococci to sulfonamides as measured by in vitro tests,[6] although this, too, is only rarely the case.[14]

Frisch and his associates also correlated the numbers of pneumococci found in Wright-stained smears of sputum with the prognosis and with other significant clinical and laboratory findings.[9, 10] He found that the numbers of pneumococci are directly proportional to the case mortality and to the incidence of bacteremia and leucopenia.

Penicillin is much more effective than sulfonamides against pneumococci in vitro and probably has a different mechanism of action. It was of interest, therefore, to study the changes in the pneumococci and other organisms in the sputum of patients under treatment with this antibiotic. The present paper deals with observations on the sputum of twenty-six patients with pneumococcal pneumonia treated with penicillin.

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MATERIALS AND METHODS

Selection of Patients.—The twenty-six patients studied were admitted to the medical wards of the Boston City Hospital between December, 1944, and April, 1945. They ranged in age between 16 and 88 years and twelve of them were over 50 years old. All were acutely ill with lobar pneumonia and produced sputum in which pneumococci were identified by Neufeld typing at the first examination. All of these patients denied previously receiving sulfonamide drugs. Blood taken on admission from twenty-two of these patients was tested for the presence of sulfonamide, and no measurable concentration was found.

Penicillin Therapy.—Penicillin therapy was begun on the third day or earlier in seventeen patients, between the fourth and sixth day in six, and on the tenth day of illness in the remaining three. The average duration of the disease at the onset of therapy was 3.5 days. Commercial penicillin (G) was given intramuscularly in most instances, but in two patients penicillin X was used. In two others oral penicillin was given, and one patient received penicillin G by inhalation. The intramuscular injection consisted of 15,000 or 20,000 units given every two hours for the first day followed by these amounts every three hours for another two or three days, after which the dose was often reduced to 10,000 units every three hours. Treatment was discontinued after three days or less in three patients, after from four to six days in seventeen, and after seven days or longer in the remaining six. The average duration of treatment was 5.7 days. The oral penicillin was given in doses of 100,000 units every two hours at first and later every three hours. The nebulized penicillin was given in doses of 20,000 units every two hours. Sulfonamides were used in only two patients for complicating infections some time after the penicillin was stopped and the sputum studies were discontinued.

Bacteriologic Studies.—Blood cultures were made on every patient before treatment was started and were repeated at irregular intervals when indicated. Sputa were obtained before the first dose of penicillin and at suitable intervals during and after treatment. These sputa were subjected to the following studies: (1) Direct examination of the sputum was made by the Neufeld method both for the different types of pneumococci and the relative numbers of each type. (2) Gram- and Wright-stained preparations were examined by the method of Frisch for the number of characteristic lancet-shaped diplococci, the presence of chaining, phagocytosis, and reticulation. (3) A characteristic portion of the sputum was homogenized, and from it streaks and substreaks were made in progression on the quadrants of the surface of horse blood agar plates in order to observe roughly the relative numbers of different bacteria, including pneumococci, beta and alpha hemolytic streptococci, staphylococci, *Hemophilus influenzae,* and *Hemophilus hemolyticus.* Identification was mostly by morphology, but all pneumococci were typed, staphylococci were tested for coagulase activity, and green streptococci were tested for bile solubility. (4) Serial tenfold dilutions in plain broth were also made from the homogenized sputum, and blood agar plates were poured with from 10^{-4} to 10^{-9} c.c. Estimations of the numbers of typical pneumococcus colonies were made,

and doubtful colonies were subcultured and identified by bile solubility and Neufeld typing. (5) Whenever pneumococci could not be identified directly in the sputum by the Neufeld method, some of the sputum was inoculated into a mouse, and the peritoneal exudate and heart blood were later examined for pneumococci.

Throat cultures were made in many instances at the time of the initial sputum and later whenever sputum was not obtainable. Streaks were made on the surface of blood agar plates from the swab or from broth into which the swab was immersed and treated like the sputum. Swabs were then used only for the study of the presence and identity of pneumococcus types.[15] Blood broth containing the swab was incubated from four to eight hours until a uniform growth occurred, after which it was examined by the Neufeld method, and a small amount was injected into a mouse. The peritoneal exudate and cultures of the heart blood were subsequently examined for pneumococci.

The sensitivity to penicillin of most of the strains of pneumococci that were isolated, and of many of the other organisms, was tested by the method of Rammelkamp and Maxon.[16]

RESULTS

A summary of certain relevant features of the cases studied in relation to the persistence of the pneumococci is given in Table I. The cases were of moderate severity; multiple lobes were involved in nine of the patients and blood cultures were positive in ten (38 per cent) at entry.

A total of 154 sputa were studied in the twenty-six patients and, in addition, forty-five throat cultures were made in twenty patients, making an average of almost eight specimens from each patient. Specimens were obtained throughout the hospital stay of the patients and, in a few instances, during follow-up visits. The last culture was made on or before the tenth day in fourteen patients, between the eleventh and twenty-first day in nine, and between the twenty-third and thirtieth day in the remaining three. The average period of observation in the twenty-four patients who recovered was thirteen days.

A total of forty-two strains of pneumococci were isolated and identified in the twenty-six cases. A single type was obtained in fourteen of the cases, two types were obtained from each of ten cases, and three and five types, respectively, were identified in the two remaining cases. Nineteen of the strains were of the types commonly associated with primary lobar pneumonia, namely types 1, 2, 4, 5, 7, and 8. Of the remaining strains, five were type 3, one was type 6, and the others were of the so-called "higher" types. In each of the twelve cases with multiple types, one of the strains of pneumococci was of the common types.

Persistence of the Pneumococci Present Before Penicillin Treatment Was Started.—The number of days during which the pneumococci present in the original sputum persisted after treatment with penicillin was started are shown in Table II. Only one of every six of these strains persisted throughout the period of observation, and twenty-one of the thirty-five strains (60 per cent)

TABLE I. OCCURRENCE AND PERSISTENCE OF PNEUMOCOCCI IN TWENTY-SIX PENICILLIN-TREATED PATIENTS WITH PNEUMONIA

CASE	BLOOD CULTURE*	LOBES INVOLVED†	PENICILLIN THERAPY: DAY BEGUN‡	PENICILLIN THERAPY: TOTAL DOSE: UNITS × 1,000§	PENICILLIN THERAPY: TOTAL DOSE: NUMBER OF DAYS	PNEUMOCOCCUS TYPE	PNEUMOCOCCI IN SPUTUM: SENSITIVITY‖	PNEUMOCOCCI IN SPUTUM: DAYS FOUND¶: FIRST	PNEUMOCOCCI IN SPUTUM: DAYS FOUND¶: LAST	PNEUMOCOCCI IN SPUTUM: LAST TEST	CLINICAL RESPONSE, COMPLICATIONS, AND REMARKS
1	7	Rl, Ll	2	515	5	7	0.016**	0	23	23	Crisis 24 hours; chronic cough
2	0	Ll	1	570	5	1	0.016	0	3	8	Lysis 3 days
3	0	Ll, Rl	10	265	1½	3	0.016	0	½	1	Died 1½ days; moribund on entry; 88 years old
4	0	Ruml, Ll	3	785	8	12	0.016	0	3	11	Lysis from 2 to 5 days; frequent colds
5	1	Ll	4	520	5	1	0.031	0	1	10	Crisis 36 hours; alcoholism
6	0	Rm	1	385	4	2	0.016	0	2	4	Crisis 48 hours
7	1	Rum	3	1,530	10	1	0.008	0	7	15	Lysis 3 days; moribund on entry; 500 colonies per cubic centimeter of blood; W.B.C. 400; polymorphonuclears, 1%; alcoholism
8	4	Rl	4	740	6	4	--	0	12	17	Lysis 4 days; chronic pyelonephritis
9	1	Ll	3	660	6	1	--	0	6	8	Lysis from 2 to 7 days; low-grade fever and pleuritic pain 12 days
10	0	Ll	3	415	5	7	0.016	0	8	8	Crisis 24 hours
11	0	Rl	2	965 X	8	28	--	0	7	7	Lysis ? 2 days; fever from active tuberculosis (positive sputum)
12	0	Ll	1	455 X	5	8	0.031	0	7	7	Crisis 12 hours
13	7	Ll	2	6,390 p.o.	6	7	0.031	0	3	12	Lysis 5 days
14	0	Ll	2	880 inh.	6	12	0.016	0	7	14	Crisis 24 hours
15	0	Rum	10	670	6	1	0.031**	0	14	14	Crisis 24 hours; temperature of 100° F. for 8 days
						43	0.016	12	12		
16	0	Ll	2	525	4	3	0.031	0	2	5	Crisis 24 hours
						20	0.031	0	5		
17	0	Rml	3	525	5	7	0.016	0	2	8	Crisis 12 hours; pneumonia (? etiology) each of last 3 winters
						6	--	2	2		
18	0	Ll	1	320	3	5	0.031	0	2	11	Crisis 24 hours
						13	--	1	1		
19	1	Ruml	6	1,950	10	1	0.031**	0	9	30	Persistent fever; ? interlobar effusion; staphylococcus (sensitivity, 8 units) persisted
						19	--	0	9		
20	0	Rl	2	500	6	3	0.008	0	4	21	Crisis 12 hours; delirium tremens
						11	0.031	4	12		
21	1	Ll	10?	450	3	1	0.031	0	½	2	Died third day; hemiplegia and coma
						9	--	0	½		
22	0	Ll, Rl	1	595	5	3	0.016	0	½	9	Lysis 3 days; "shock" on entry; asthma; bronchiectasis; congestive heart failure
						14	--	0	0		
						44	0.016	0	2		
23	1	Ll	4	425	4	1	0.016	0	4	6	Crisis 24 hours; alcoholism
						8	--	1	1		
						18	0.016	1	1		
						19	0.031	0	1		
						44	0.016	0	½		
24	0	Rl	6	940	9	3	0.008	0	½	25	Lysis 3 days; chronic cough
						17	0.016	0	2		
25	0	Ll	1	555	4	8	0.016	0	1	9	Crisis 24 hours; "pleurisy" 2 years before
						20	0.016	2	2		
26	7	Ruml	5	8,505 p.o.	9	7	0.008	0	2	19	Lysis from 2 to 5 days; ? interlobar effusion
						19	--	0	1		

*Numbers represent types of pneumococci and 0, no growth.
†R, Right; L, left; u, upper; m, middle; l, lower.
‡Refers to day of disease.
§X, Intramuscular penicillin X; p.o., oral; inh., by inhalation; others received intramuscular commercial penicillin.
‖Minimum concentration of penicillin (units per cubic centimeter) required to sterilize a culture inoculated with from 1,000 to 10,000 pneumococci per cubic centimeter in eighteen hours; --, not done.
¶Refers to day after onset of treatment; 0, specimen obtained before treatment was given.
**Same result in last specimen.

TABLE II. PERSISTENCE OF THIRTY-FIVE STRAINS OF PNEUMOCOCCI FOUND BEFORE PENICILLIN TREATMENT WAS STARTED

LAST POSITIVE CULTURE	NUMBER OF STRAINS
First day	11
Second day	7
Third day	3
Fourth day	2
Sixth to twelfth day	6
Total	29 (83%)

Average persistence of all thirty-five strains, 4.3 days.

Average persistence of the twenty-nine strains which disappeared during the period of observation, 3.0 days.

TABLE III. SIX STRAINS OF PNEUMOCOCCI WHICH PERSISTED THROUGHOUT PERIOD OF OBSERVATION

CASE	DAYS OF PENICILLIN THERAPY	PNEUMOCOCCUS TYPE	PENICILLIN SENSITIVITY	DAYS POSITIVE	COMPLICATIONS
1	5	7	0.016*	23	Chronic bronchitis
10	5	7	0.016	8	
11	8	28	----	7	Tuberculosis (sputum positive)
12	5	8	0.031	7	
15	6	1	0.031*	14	
16	4	20	0.031	5	

*Same for original strain obtained before treatment and for the strain obtained twelve days later.

were found for only three days or less. The average time that elapsed between the beginning of penicillin treatment and the last time these pneumococci were found was 3.0 days. Twenty-six of the twenty-nine strains could no longer be identified in sputum or throat cultures before the penicillin therapy was completed.

A summary of the relevant findings concerning the six strains which persisted throughout the period of observation is shown in Table III. Five different types were represented. Treatment with penicillin was given for from four to eight days in these cases and they were studied for from five to twenty-three days (average 10.7 days). The strains were similar in penicillin sensitivity to those which disappeared early. In two instances strains of the same type isolated before treatment and again twelve days later were compared, and no change in sensitivity to penicillin could be demonstrated. Two of the patients from whom these strains were obtained had chronic respiratory infections. In three of the patients the strains of pneumococci had disappeared temporarily only to reappear in later cultures.

Appearance of Additional Strains of Pneumococci During or After Penicillin Therapy.—Seven strains of pneumococci appeared in six patients after penicillin therapy was started, five during the treatment and two after it was stopped. Six of these strains were identified in only one specimen and the remaining one was first noted on the fourth day of treatment and persisted over an eight-day period. None of them was present in the last culture. One of the strains was type 8, and the others were of higher types. The penicillin sensitivity of the new strains was compared with that of the original ones in four

TABLE IV. PENICILLIN SENSITIVITY OF STRAINS OF PNEUMOCOCCI AT TIME OF THEIR ORIGINAL ISOLATION

MINIMUM INHIBITING CONCENTRATION OF PENICILLIN	NUMBER OF STRAINS	NUMBER OF STRAINS WHICH PERSISTED THROUGHOUT THE PERIOD OF OBSERVATION
0.008 units per cubic centimeter	4	0
0.016 units per cubic centimeter	17	2
0.031 units per cubic centimeter	11	3
Total	32	5

cases. These were found to be the same in two instances (Cases 23 and 25); the new strain was twice as sensitive as the original one in Case 15, and the original strain was four times as sensitive as the new one in Case 20.

Sensitivity of the Pneumococci to Penicillin.—Thirty-two strains of pneumococci were isolated when first encountered and were tested for sensitivity to penicillin. Twenty-eight of these strains were obtained before treatment was started, three were isolated during penicillin therapy, and one was isolated after it was discontinued. The results are shown in Table IV. All were quite susceptible, most of them requiring two or four times as much penicillin as a control strain of hemolytic streptococcus, No. 98, which was sensitive to 0.008 units per cubic centimeter of culture. Three additional strains of the same type were isolated again, one after four days of treatment and the other two after eight days, and all were just as sensitive as the original strains. The strains isolated during or after therapy were within the same range of sensitivity as those isolated before treatment was started. There is a slight suggestion that the less sensitive strains tended to persist longer, but the number of strains and the range of their sensitivity were too small for any definite conclusion.

Clinical Response to Treatment.—Among the patients who recovered, twelve were essentially afebrile and free of acute symptoms within forty-eight hours after the first dose of penicillin, while in nine others some fever persisted for from three to five days, although the patients had shown symptomatic improvement earlier. In three patients low-grade fever persisted for eight, twelve, and twenty-eight days, respectively; the first two of these patients had no complications, but the third developed a staphylococcal pneumonia and probably some interlobar effusion.

There were two deaths. One occurred (Case 3) in a man 88 years old with type 3 pneumococcal pneumonia involving both lower lobes. The blood culture taken on the tenth day of the disease and before treatment was started was negative. Pneumococci could not be found in the last sputum which was taken twenty-four hours after penicillin was started, and the patient died twelve hours later. The second patient (Case 21) was 65 years old and had a type 1 pneumococcal pneumonia and bacteremia. He became comatose, developed a hemiplegia, and died on the third day. Pneumococci could not be found in the throat culture taken shortly before death.

A summary of the persistence of pneumococci in relation to the duration of fever after treatment was started is shown in Table V. There was no defi-

TABLE V. RELATION OF RAPIDITY OF CLINICAL RESPONSE AND PERSISTENCE OF PNEUMOCOCCI FOUND BEFORE TREATMENT WAS STARTED

DAYS SPUTUM POSITIVE	RECOVERED WITHIN 48 HOURS	FEVER FROM 3 TO 5 DAYS	FEVER 8 DAYS OR MORE	DIED
1 to 3	7	11	--	3
4 to 10	7*	1	3	-
More than 10	1†	1	1‡	-
Strains	15	13	4	3
Cases	12	9	3	2

*Last culture positive on fifth day.
†Last culture positive on twenty-third day.
‡Last culture positive on fourteenth day.

TABLE VI. RELATION OF BACTEREMIA TO PERSISTENCE OF PNEUMOCOCCI UNDER PENICILLIN TREATMENT

DAYS SPUTUM POSITIVE	NUMBER OF CASES WITH BLOOD CULTURE	
	POSITIVE	NEGATIVE
3 or less	4	9
4 to 10	4	6†
More than 10	2*	1‡
Total cases	10	16

*Last culture positive on twenty-third day in one case.
†Last culture was positive in four cases.
‡Last culture positive on fourteenth day.

nite correlation except among the three cases in which the fever continued the longest. Among these the pneumococci persisted more than three days in each instance.

Bacteremia.—There were ten patients in whom positive blood cultures were obtained before penicillin therapy. Type 1 pneumococci were obtained in six patients, type 7 in three, and type 4 in one. The persistence of pneumococci in these patients compared with that in the patients with negative cultures is shown in Table VI. There is a suggestion of a longer persistence of pneumococci in the sputa of the patients with bacteremia, but the numbers of these patients are too small and the correlation is not striking. Case 7 is of interest in this respect. The patient had an overwhelming bacteremia with about 500 colonies of type 1 pneumococci grown per cubic centimeter of blood before treatment, and the leucocyte count at the time was 400 per cubic millimeter of blood, of which only 1 per cent were polynuclear cells. This patient showed steady and dramatic improvement under penicillin therapy. The second blood culture taken after ten hours was sterile, but pneumococci persisted in the sputum for seven days and could not be found thereafter.

Effect of Chronic Respiratory Infections and Previous History of Pneumonia.—There were five patients who gave a history of chronic cough of more than a few months' duration. In only one of them (Case 1) did pneumococci persist after treatment. Five additional patients had other chronic respiratory complaints: one had frequent upper respiratory tract infections, one had asthma, one had recurrent pleurisy, one had active pulmonary tuberculosis, and one had an attack of pneumonia each of the three preceding winters. The only one of these five patients in whom pneumococci persisted during seven days of

TABLE VII. RELATION OF CHRONIC RESPIRATORY SYMPTOMS TO PERSISTENCE OF PNEUMOCOCCI IN SPUTUM AFTER PENICILLIN THERAPY

DAYS SPUTUM POSITIVE	PATIENTS WITH CHRONIC RESPIRATORY SYMPTOMS	PATIENTS WITHOUT CHRONIC RESPIRATORY SYMPTOMS
3 or less	7	6
4 to 10	2*	8‡
More than 10	1†	2§
Total cases	10	16

*Last culture positive seventh day in one patient.
†Last culture positive twenty-third day.
‡Lost culture positive in three patients.
§Last culture positive in one patient.

observation was the one with active tuberculosis (Case 11). Pneumococci were found after the third day in only one additional patient. A comparison of the persistence of pneumococci in the patients with and without chronic respiratory symptoms is given in Table VII. There was no correlation in this small group of patients.

Effect of Complications.—As might be expected in penicillin-treated cases, purulent pneumococcal complications were not recognized in the present cases. One patient (Case 19) developed a complicating staphylococcal pneumonia and signs of an effusion. Signs of a small interlobar effusion were also made out in another patient (Case 26), but the presence of fluid was not proved by thoracentesis in either of these two patients. The occurrence of agranulocytosis before treatment was started in Case 7 has already been mentioned, and this patient also suffered from alcoholism and a toxic psychosis. Pneumococci persisted for nine, two, and seven days, respectively, in these three patients.

Significance of the Numbers of Pneumococci in the Initial Sputum.—Quantitative bacteriologic studies of sputum and throat cultures are notoriously unsatisfactory and involve tremendous errors. When a single organism is greatly predominant or is present in almost pure culture, as is sometimes the case in severe pneumonia, such studies may be carried out successfully. When a variety of organisms are found in relatively large numbers, as they usually are after therapy with active chemotherapeutic or antibiotic agents, the quantitative aspects are not very reliable. Only a rough estimation then can be made of the relative numbers of the different organisms present and even this involves considerable errors. In general, it has not been possible to differentiate the individual colonies of organisms in poured blood agar plates of sputum containing more than 10^{-5} c.c., and more often 10^{-6} or 10^{-7} c.c. was the largest amount that proved workable. Even smaller amounts must sometimes be used if individual colonies are to be picked and identified.

In Table VIII is listed the number of patients in whom from two to ten pneumococcus colonies were recognized in pour plates of various dilutions of the original sputum obtained before penicillin was started. This is compared with the numbers of pneumococci seen in Wright- and Gram-stained smears of the same sputum and is also correlated with some of the more important features of the disease. The number of cases is too few for any definite deductions. It does seem, however, that there was a fairly good correlation between

TABLE VIII. ROUGH QUANTITATION OF NUMBERS OF PNEUMOCOCCI IN SPUTUM OBTAINED BEFORE PENICILLIN THERAPY CORRELATED WITH CERTAIN IMPORTANT FEATURES OF PNEUMONIA

GREATEST DILUTION SHOWING PNEUMOCOCCI	NUMBER OF CASES	PNEUMOCOCCI PER HIGH-POWER FIELD IN WRIGHT-STAINED SMEARS			CASES WITH POSITIVE BLOOD CULTURES	MULTIPLE LOBE INVOLVEMENT	LEUCOCYTES PER CUBIC MILLIMETER		CLINICAL RESULT		
		MORE THAN 100	50 TO 100	LESS THAN 50			5,000 TO 10,000	LESS THAN 5,000	CRISIS LESS THAN 48 HOURS	LYSIS 3 DAYS OR MORE	DIED
10^{-9}	3	3	0	0	2	2	2	1	0	2	1
10^{-8}	11	3	1	7	4	4	2	2	6	4	1
10^{-7}	10	1	1	8	4	3	2	0	5	5	0
10^{-6} or 10^{-5}	2	0	0	2	0	0	1	1	1	1	0
Total	26	7	2	17	10	9	7	4	12	12	2

the number of pneumococci per cubic centimeter of sputum as determined in the pour plates and the number of characteristic diplococci seen per high-power field in the Wright-stained preparations. Little can be said of the other features. Bacteremia, multiple lobe involvement, and leucopenia tended to occur in patients with large numbers of pneumococci, but this does not show very clearly from the present data. The two patients who died had large numbers of pneumococci, although the deaths were associated with other factors. Following treatment, the pneumococci which persisted were found only in the pour plates of smaller dilutions, and very few were seen in the smears.

The relation of the number of pneumococci observed in the original sputum and their persistence after treatment is roughly shown in Table IX. Within the limits of the method, there seemed to be little correlation between the numbers of pneumococci counted in the pour plates and their persistence except that the patients in whom the organisms persisted for more than ten days all originally had large numbers. The correlation between the numbers of pneumococci seen in the original smears and the persistence of pneumococci was somewhat better but still not very striking.

TABLE IX. PERSISTENCE OF PNEUMOCOCCI IN RELATION TO NUMBERS FOUND BEFORE THERAPY

APPROXIMATE NUMBER OF PNEUMOCOCCI	NUMBER OF DAYS PNEUMOCOCCI PERSISTED IN THE SPUTUM			TOTAL NUMBER OF CASES
	3 OR LESS	FROM 4 TO 10	MORE THAN 10	
Per cubic centimeter of sputum:				
10^9	1	1	1	3
10^8	6	2	3	11
10^7	6	4	0	10
10^6 or 10^5	–	2	–	2
Per high-power field (Smears):				
More than 100	3	2	2	7
From 50 to 100	–	1	1	2
Less than 50	10	6	1	17
Total cases	13	9	4	26

Recognition of Pneumococci.—Throughout this study there was no difficulty in recognizing pneumococci and identifying their type regardless of whether they were found before treatment was begun, during penicillin therapy, or after it was discontinued. When sputum was available and pneumococci were present in the cultures, they were also usually identified directly in the sputum by the Neufeld method, characteristic colonies were observed on the surface of blood agar plate cultures, and the sputum caused the death of mice with multiplication of the organisms in the peritoneal exudate and invasion of the blood stream. Quantitative tests for the virulence of the pneumococci for mice were not done.

Occurrence of Phagocytosis, Chaining, and Morphologic Alterations of the Pneumococci.—In the present studies no definite relationship was noted between the occurrence of chaining and phagocytosis of the pneumococci in the stained smears of the sputum and the duration of the disease or of penicillin treatment or the persistence of pneumococci in the sputum. Chaining was observed in only eight cases, was transient, and was not prominent in any of the specimens.

Phagocytosis was marked in only a few instances. Phagocytosis was first noted between the first and seventeenth day of the disease:—on or before the third day in ten patients, between the fourth and seventh days in eight, on the eighth day or later in five, and not at all in three. In fourteen patients the phagocytosis was first noted before penicillin therapy was started; in six it was seen on the first day of treatment; in two others it was first observed on the third or fourth day of therapy; and in one patient it was noted only eight days after treatment was stopped.

Some morphologic changes in the pneumococci were observed in the smears made after penicillin had been given. In several instances the pneumococci assumed bizarre shapes, took the stain poorly, and appeared to be fragmented in the Wright- or Gram-stained smears of sputum. Similar changes in bacteria subjected to the action of penicillin have been noted by Gardner,[17] and they have also been seen in smears of empyema fluid after local penicillin instillation.[18]

Reticulation, as described by Frisch,[5] was noted in Wright-stained preparations of three of the type 3 cases, in two type 7 cases, and in one type 8 case. It was not marked in any of these cases. The last sputum culture was positive in each of the type 7 and type 8 cases but not in the type 3 cases.

Occurrence of Organisms Other Than Pneumococci.—During penicillin therapy the number of organisms which grew in pour plates of sputum, or on the surface of blood agar plates streaked with sputum, characteristically declined. A few colonies of *Staphylococcus albus* and moderate numbers of alpha hemolytic or nonhemolytic streptococci persisted in almost every case. In twenty-one of the cases small Gram-negative bacilli having the morphology and colony characteristics of *H. influenzae* appeared and usually became numerically the predominating organisms during the course of therapy. *H. hemolyticus* appeared in the sputum of ten patients but did not become predominant in any of them. Beta hemolytic streptococci were found in only five patients, usually in very small numbers and before treatment was started. They disappeared rapidly and completely soon after penicillin was given. Friedländer's bacillus, type A, was present in the sputum of one patient.

Case 19 is an interesting one from the point of view of the occurrence and fate of different types of organisms in the course of therapy. On admission, type 1 pneumococci were grown in the blood culture and types 1 and 19 pneumococci were found in the sputum. The pneumococci disappeared gradually from the sputum after nine days of penicillin therapy. A coagulase-positive and hemolytic *Staphylococcus aureus* first appeared twelve hours after penicillin was started and became the predominant organism. It persisted throughout the hospital course, although there was a definite reduction in the numbers of this organism after sulfadiazine was started. *H. influenzae* also appeared and persisted but in smaller numbers than the staphylococcus. The penicillin sensitivity of the type 1 pneumococcus in this case was 0.031 unit per cubic centimeter, that of the influenza bacillus was 0.5 unit per cubic centimeter, and that of the staphylococcus was from 4 to 10 units per cubic centimeter. The staphylococcus was isolated and tested for sensitivity to penicillin on four

separate occasions with similar results each time. It is of interest, therefore, that in this patient the pneumococcus, which was the most sensitive to penicillin, disappeared first, and the staphylococcus, which was the most resistant of the organisms, persisted the longest.

The penicillin sensitivity of some of the strains isolated in the course of this study have been included in a previous report.[19] Of special interest were seven strains of *H. hemolyticus* which were similar in penicillin sensitivity to most meningococci and staphylococci. The most resistant of these strains required 0.25 unit per cubic centimeter. One strain was sensitive to 0.125 unit, four were sensitive to 0.063 unit, and one was sensitive to 0.031 unit per cubic centimeter.

DISCUSSION

It is appreciated that the number of patients available for the present study was much smaller than was desired. The marked general reduction in the incidence of pneumococcal pneumonia, coupled with the difficulty of finding such patients who arrive in a general hospital before they have been given some antibacterial therapy, is in large part responsible for the paucity of cases. Furthermore, the follow-up in these patients was relatively short due chiefly to the rapid improvement in their clinical condition and consequently their brief period of hospitalization. The cough and sputum in these patients cleared up quite rapidly, although low-grade fever often persisted for several days.

There was little difference in the bacteriologic response of the patients treated with penicillin and those previously studied in this clinic under sulfonamide therapy.[14] Penicillin acts somewhat more rapidly than do the sulfonamides in vitro, but in the patients the two agents seem to cause disappearance of pneumococci from the sputum with equal rapidity. (The pneumococci did, however, clear more rapidly from the sputum after penicillin.[20]) The differences were not very striking in this small group of patients. Penicillin appears to be more effective than the sulfonamides in preventing purulent pneumococcal complications, and the same mechanism may also have resulted in a smaller percentage of patients in whom pneumococci persisted under penicillin treatment. Only six strains, or 17 per cent of the thirty-five which were originally present, persisted after penicillin therapy, as compared with 30 per cent among the sulfonamide-treated patients previously studied in this clinic.[14] A similar proportion, namely three-fourths of the strains which cleared during therapy, had already disappeared by the third day of treatment with either agent.

The dosage used in the present series of patients was considered to be adequate although others may consider it only moderate. It was sufficient to maintain levels of penicillin in the blood above those required by the sensitivity of the pneumococci, and it seemed adequate clinically. It is possible, however, that larger doses which may permit greater penetration into infected foci might have cleared the pneumococci more rapidly and more completely. The possibility is further suggested by the results obtained in beta hemolytic streptococcal respiratory infections in which the organisms are more sensitive to penicillin and in which similar doses result in more rapid clearing of the

organisms from the pharynx.[21] The reason for the failure of pneumococci to disappear from the sputum in some patients, in spite of their susceptibility to penicillin, is not entirely clear but probably depends on their being protected in some way from the action of the antibiotic.

In the present series the persistence of pneumococci could not be clearly related to bacteremia, to underlying respiratory disease, to resistance of the organisms to penicillin, to the numbers present before treatment, or to the failure of the antibody mechanism, at least as judged from the presence of phagocytosis in the sputum smears. The failure to observe such correlations may be due in part to the small number of patients, but it is more likely a result of the relatively great effectiveness of penicillin as an antipneumococcal agent.

It is highly probable that most of the pneumococcus strains which were first recognized after penicillin therapy was started were actually present before that time but in too small numbers to be recognized among the other pneumococci which predominated. They were almost all found during the first day or two of treatment and disappeared quite rapidly.

The changes in the flora of the sputum under penicillin therapy is also of interest. Gram-negative bacilli replaced the more susceptible gram-positive cocci and predominated in most instances after recovery. The complicating staphylococcal pneumonia represents an example of a patient in whom a virulent but penicillin-resistant organism becomes predominant and replaces the original infecting organism. In this patient the staphylococcus was clinically more susceptible to sulfadiazine than to penicillin. It is of interest that in the two patients who died the pneumococci cleared rapidly and death in each case was due to other immediate causes.

SUMMARY

Sputum and throat cultures and stained smears of sputum were studied before, during, and after treatment with penicillin in twenty-six patients with pneumococcal pneumonia. Considerable differences were noted in the persistence of pneumococci in these patients. About one-sixth of the strains found before penicillin therapy was started persisted throughout the period of observation. About three-fourths of the remaining strains could no longer be found after the third day of treatment.

Strains of pneumococci of types not identified in the pretreatment cultures were found for the first time during or after penicillin therapy in six patients. Only one of the strains, however, was found in more than a single specimen.

All of the strains of pneumococci tested were sensitive to penicillin, and three strains isolated after therapy were found to be just as sensitive as the pretreatment strains of the same types isolated from the same patients.

The persistence of pneumococci in the sputum did not appear to be definitely related to bacteremia, complications, chronic respiratory infections, the numbers of organisms present before treatment, the development of penicillin resistance, or the failure to develop antibodies, as judged by phagocytosis of pneumococci in stained smears.

In one patient a staphylococcal pneumonia developed as a complication of the pneumococcal lobar pneumonia. The staphylococcus first appeared in the culture of the sputum obtained twelve hours after penicillin was started and then became predominant in subsequent cultures. It was quite resistant to penicillin, and improvement occurred only after intensive sulfonamide therapy.

Following penicillin therapy, the flora of the sputum changed in most instances to one in which the predominant organisms were gram-negative bacilli having the morphology and colony characteristics of influenza bacilli.

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PRODUCTION OF ANEMIA IN A PIG WHICH RESPONDED TO PURIFIED LIVER EXTRACT

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CASTLE and co-workers[1] demonstrated that several commercial crude casein preparations possessed significant "extrinsic factor" activity and that careful purification was required for the elimination of the extrinsic factor. Borden's Labco vitamin-free casein was found to be essentially free of such activity. Since our swine experiments[2] were performed with crude casein, it seemed desirable to ascertain the effect of the substitution of Labco vitamin-free casein for the crude Sheffield new process casein used, thus eliminating the extrinsic factor from the diet. The following experiment was performed with this in mind but was carried out only in one animal because of the great cost of the "vitamin-free" casein. The animal was also given sulfasuxidine in the diet. An anemia developed which responded to purified liver extract.

Recently it has been demonstrated in three different laboratories[3-5] that synthetic "folic acid" (*Lactobacillus casei* factor from liver) has antipernicious anemia activity. Because of this fact and its significance in relation to the causation of the nutritional anemias, it was decided to put our observations in a single animal on record at this time. We are well aware that observations in one animal are not sufficient to permit one to draw definite conclusions. We are also aware of the fact that we have not proved that this animal was deficient in "folic acid." However, because the high cost of the experiment prohibits its being repeated in a large series of animals and because of the interest in experimentally induced anemias at the present time, it was felt that it would be worth while to make our results known since they are clear cut and appear to be unique.

METHODS

A pure-bred Duroc pig, 30 days of age, was placed on a basal diet consisting of Borden's Labco vitamin-free casein, 26.1 per cent; sucrose, 57.7 per cent; lard, 11.0 per cent; salt mixture (swine salt mixture No. 3[2]), 5.2 per cent. Sulfasuxidine* was added to the diet in an amount of 2.0 per cent. In addition, the animal received blended oil (1,800 units of vitamin A, 175 units of Vitamin D per gram),† 0.5 Gm. per kilogram of body weight per day. Vitamins‡ were supplied in crystalline form by placing them in capsules and administering them orally. The quantities of crystalline vitamins given were as

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‡Kindly furnished by Merck & Co., Inc., New York, N. Y., through the courtesy of Dr. D. F. Robertson.

follows (milligrams per kilogram of body weight per day): thiamine hydrochloride, 0.25; riboflavin, 0.12; nicotinic acid, 1.20; pyridoxine hydrochloride, 0.20; calcium pantothenate, 0.50; p-aminobenzoic acid, 0.50; inositol, 1.20; choline, 10.0. Full details of the experimental methods have been given elsewhere.[2]

Plasma iron determinations were made by the method of Kitzes, Elvehjem, and Schuette.[6] The method of Grinstein and Watson[7] was followed for the determination of erythrocyte protoporphyrin. Serum copper was measured according to the procedure of Cartwright, Jones, and Wintrobe.[8]

RESULTS

After about sixty-five days, thinning of the hair over the rump and back was noted. The animal began to develop an untidy appearance. By about ninety days there was almost complete denudation over the flanks and rump and thinning of hair elsewhere (Fig. 1). Black crusts appeared about the eyelids (Fig. 2). There was a definite impairment of growth (Fig. 3). At 170 days of age the animal weighed 29 kilograms as compared with a normal of 72 kilograms. The gait at all times was not remarkable.

On the one hundred twentieth day of the experiment 1 mg. of biotin* was given intramuscularly and this therapy was continued for seventeen days. There was no change in the general appearance, coat, growth (Fig. 3), or blood (Fig. 4) of the animal. On the one hundred forty-first day of the experiment the volume of packed red cells had fallen to 21 c.c. per 100 c.c. The anemia was normocytic and very slightly, perhaps not significantly, hypochromic. The erythrocyte protoporphyrin (Fig. 4) was in the lower range of normal for pigs. The plasma iron, which had previously been elevated, had fallen to normal. The serum copper was definitely elevated. Unfortunately neither a white cell nor platelet count was done at this time. On the one hundred twentieth day of the experiment, at which time no anemia was present, the white cell count was 17,400 and the platelet count 370,000.

Purified liver extract,† 1 c.c. intramuscularly, was administered on the one hundred forty-first day and this dosage was continued for the next nine days. By the fifth day there was a definite reticulocyte response which reached a maximum of 9.4 per cent on the tenth day of therapy (Fig. 4). There was an immediate rise in hemoglobin and volume of packed red blood cells. The plasma iron after some fluctuation rose to the previous high level. The serum copper slowly fell until it reached the normal range. There was a slight transient rise in the erythrocyte protoporphyrin during the reticulocytosis. Following the initial response there was a gradual increase in the hemoglobin and volume of packed red cells to the lower range of normal and a marked resumption of growth took place. Fifty days after the liver therapy the animal had gained 30 kilograms of weight, a new complete and glossy coat of hair had appeared, and the animal, except for a moderate stunting of growth, was normal.

*Kindly furnished by Merck & Co., Inc., New York, N. Y., through the courtesy of Dr. D. F. Robertson.

†Parke, Davis & Co., 15 units per cubic centimeter.

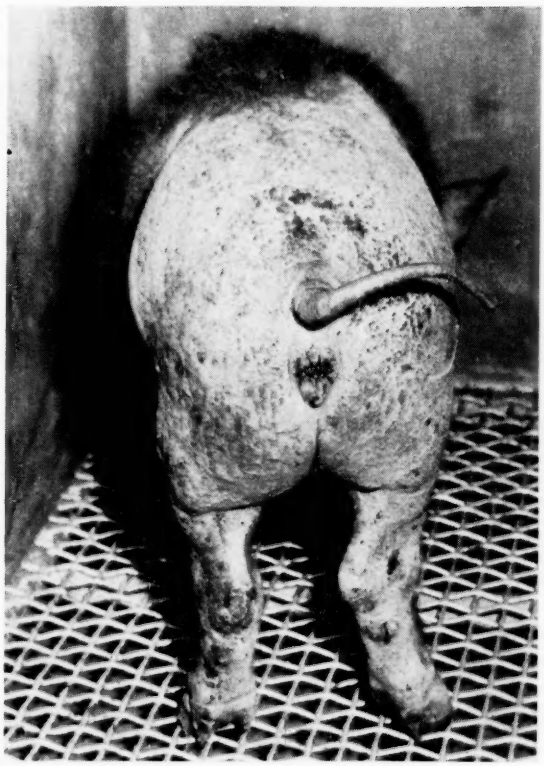

Fig. 1.—Marked alopecia, especially in the hind quarters of a pig fed vitamin-free casein and sulfasuxidine.

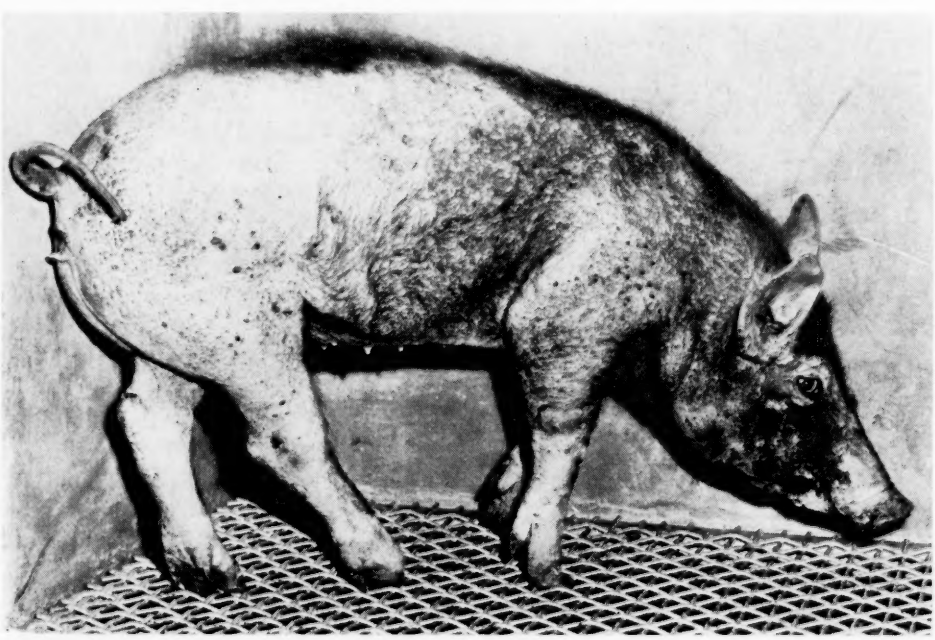

Fig. 2.—Same animal as shown in Fig. 1. Note the black crusts about the eyelids.

To determine whether or not these results could be reproduced by feeding sulfasuxidine in the diet in the presence of crude casein rather than purified casein, four animals were given the same diet as this animal received except that Sheffield's "new process" casein was substituted for Borden's Labco

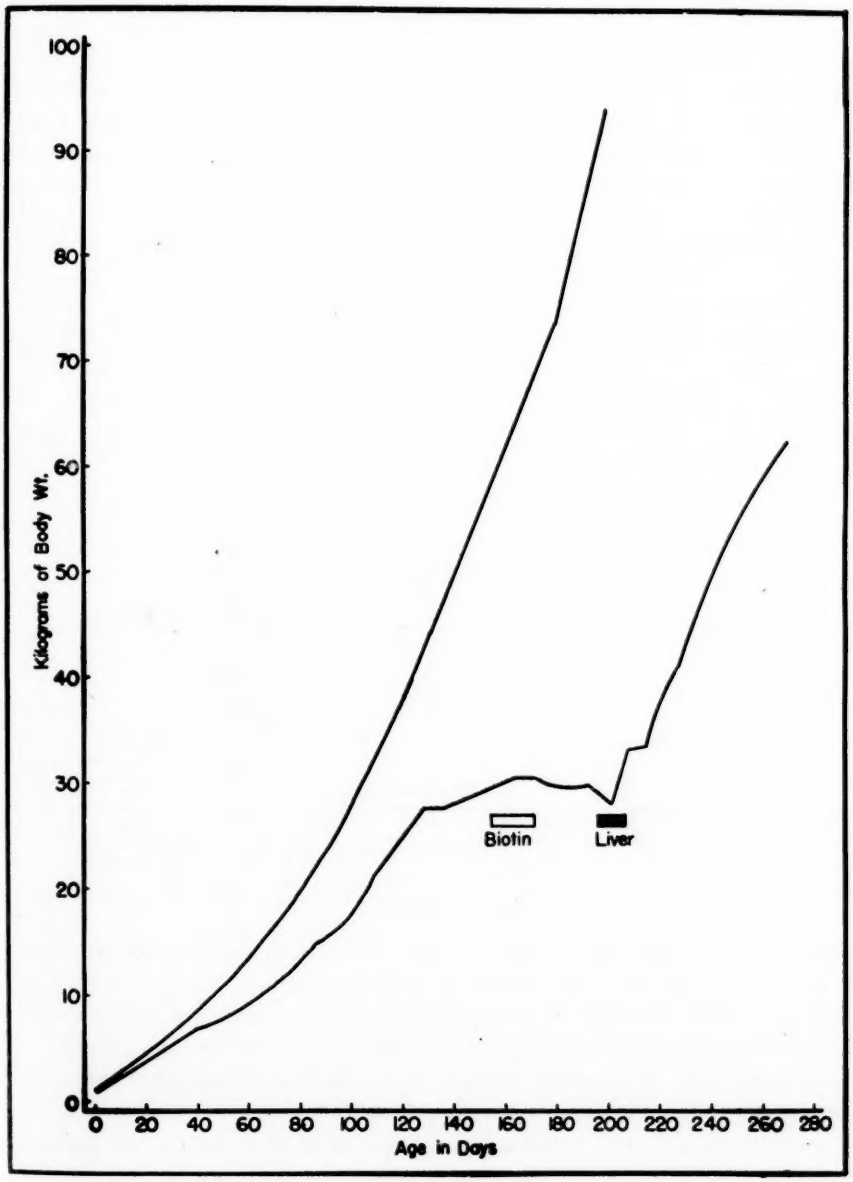

Fig. 3.—Comparison of the growth of the pig shown in Figs. 1 and 2 with that of an animal given crude casein and no sulfasuxidine. Note the failure of biotin therapy and the prompt growth response to the injection of liver extract.

vitamin-free casein. After seven months on this regimen no anemia had developed, the hair was abundant and of good texture, and there was no evident impairment of growth.

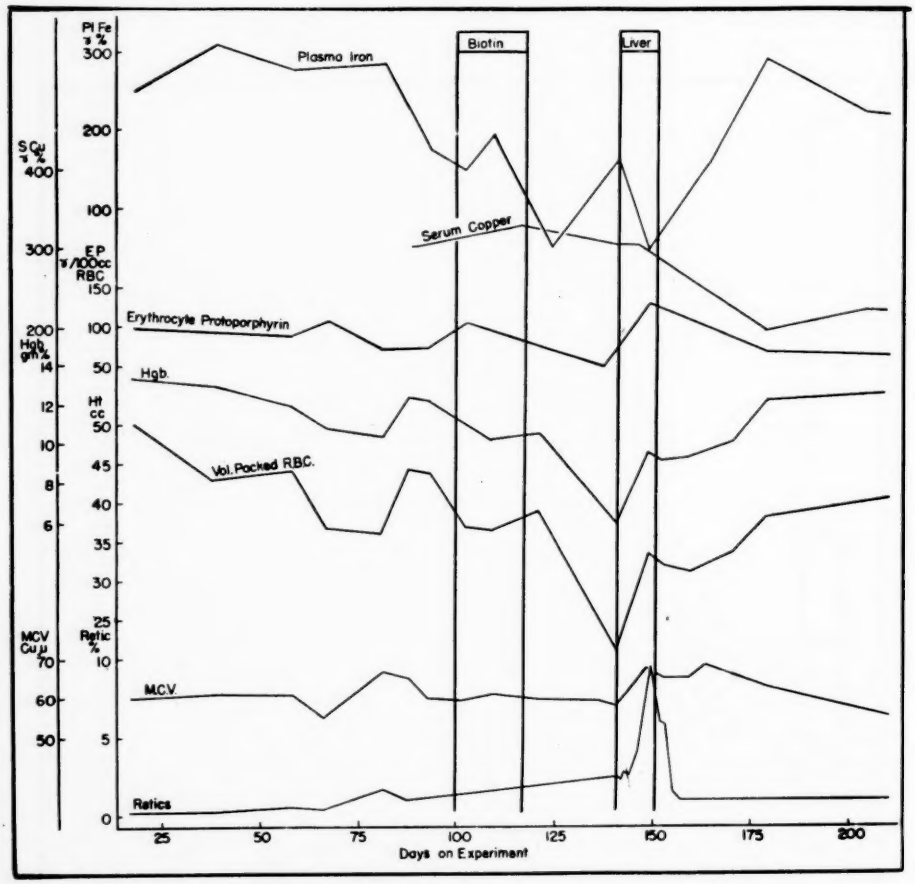

Fig. 4.—Changes in the blood of the animal given crude casein and no sulfasuxidine. Note the reticulocyte response and the relief of anemia following liver therapy.

COMMENT

An anemia developed in this animal which responded to highly purified liver extract. Whether the anemia occurring in this animal was the counterpart of pernicious anemia in human beings we are unable to state. The diet used in this experiment presumably contained no "extrinsic factor." As in pernicious anemia, the serum copper was high and the erythrocyte protoporphyrin normal. Pernicious anemia, however, is macrocytic and is usually accompanied by elevated plasma iron, whereas in this animal the anemia was normocytic and the plasma iron was normal.

In our animal the anemia was produced in the same fashion as "folic acid" deficiency anemia is induced in rats.[10] Borden's Labco casein contains very little "folic acid."[12] Since the administration of "folic acid" (*L. casei*

factor) produces a hemopoietic response in pernicious (macrocytic) anemia in man,[3-5] it is interesting that "folic acid" deficiency in chicks is macrocytic,[9] whereas in rats[10] and monkeys[11] a similar deficiency gives rise to a normocytic anemia. It is possible that in the pig "folic acid" deficiency is associated with the development of normocytic anemia.

SUMMARY

One pig maintained on a diet in which purified casein (Borden's Labco vitamin-free) was substituted for crude casein (Sheffield new process) and to which 2 per cent sulfasuxidine was added failed to grow normally and developed partial alopecia and a normocytic anemia. Following treatment with a highly purified antipernicious anemia liver extract, body hair growth was resumed, and following a definite although not marked reticulocytosis the blood returned to normal. The anemia was accompanied by normal erythrocyte protoporphyrin, normal plasma iron, and an elevated serum copper level.

Four animals maintained on a diet in which crude casein was used in place of the purified casein and to which sulfasuxidine was added grew well, maintained normal coats, and failed to develop anemia.

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THE RELATION OF THE PLASMA SALICYLATE LEVEL TO THE DEGREE OF HYPOPROTHROMBINEMIA

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INTEREST has been renewed in the use of massive doses of sodium salicylate for the treatment of rheumatic fever since the report by Coburn[1] of results with this form of therapy not attained by the use of smaller doses. It has become apparent, however, that large doses of this drug produce hypoprothrombinemia somewhat analagous to that caused by dicumarol. In this study, plasma salicylate levels and prothrombin determinations were performed on a number of patients and rabbits receiving large doses of sodium salicylate. It seemed worth while to determine whether the degree of hypoprothrombinemia might be correlated with the total daily dose of sodium salicylate or with the level of salicylate in the plasma. Elsewhere we have indicated that many clinical manifestations of salicylate intoxication may be correlated closely with the height of the plasma salicylate level which in a given individual may be difficult to predict from the dosage employed.[2] Actually the salicylate content of the plasma of one individual may be greater than that of another individual who is receiving twice as much of this drug per kilogram of body weight. In previous studies attempts to correlate hypoprothrombinemia with salicylate medication have usually referred to the total salicylate dose rather than to the plasma salicylate level.[3-5] In a few instances, the effect of large doses of synthetic vitamin K was observed in man and animals in our studies.

There is abundant clinical evidence that sodium salicylate and other salicylate derivatives may produce hemorrhage into the gastrointestinal tract and elsewhere. Instances of such complications prior to 1940 have been reviewed by Link and co-workers[3, 6] and will not be discussed here. More recently additional cases with hemorrhagic manifestations have been observed in individuals with salicylate intoxication.[7, 8]

Link and co-workers[3] have demonstrated the occurrence of hypoprothrombinemia after a single oral or intravenous dose of salicylic acid to rats which were maintained on a diet deficient in vitamin K. If the diet were adequate in vitamin K or supplemented by synthetic vitamin K, hypoprothrombinemia could be prevented. Rapoport, Wing, and Guest[9] were able to produce hypoprothrombinemia in normal rabbits after a single injection of methyl salicylate.

Many reports are now available which indicate that single or repeated doses of sodium salicylate or other salicylate derivatives, in suitable amounts, result in hypoprothrombinemia in man.[4, 5, 9-12] Of interest in this regard are

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the observations of Shapiro and co-workers[4, 5] who reported that hypoprothrombinemia usually can be prevented if synthetic vitamin K is administered with the salicylate medication. Most of their patients who were receiving 6 Gm. of acetylsalicylic acid daily developed no hypoprothrombinemia if they also received 6 mg. of vitamin K daily. However, hypoprothrombinemia occurred in one patient on this regime and disappeared after daily intravenous infusions of cevitamic acid were given. Meyer and Howard[13] also found that small doses of vitamin K would prevent the hypoprothrombinemic effect of salicylates. Fashena and Walker[10] commented on the efficacy of vitamin K in causing the disappearance of prothrombinopenia in salicylate intoxication.

MATERIAL AND METHODS

The clinical material consisted of cases of acute rheumatic fever and active rheumatoid arthritis treated with large doses of sodium salicylate. These patients received an ordinary hospital ward diet without added vitamins. The prothrombin times and the salicylate levels of the plasma were determined repeatedly in six children and eighteen adults. The sodium salicylate was given intravenously or orally. The range of dosage was from 0.06 to 0.28 of a gram per kilogram of body weight per day in the adults (from 5.4 to 18 Gm.) and was from 0.11 to 0.31 of a gram per kilogram of body weight in the children.

Similar studies of prothrombin times and salicylate levels of the plasma were made in adult rabbits (mostly female) which ranged in weight from 2.2 to 3.4 kilograms. Administration of sodium salicylate was attempted orally, intraperitoneally, intravenously, and subcutaneously. In our hands, the most satisfactory route appeared to be the subcutaneous one, and this was employed exclusively in the animals to be considered. Sodium salicylate in the form of an isotonic solution was given subcutaneously in divided doses at eight-hour intervals for periods ranging from two to thirty days. The total daily dose varied from 0.15 to 1.0 Gm. per kilogram in different animals. As in the case of patients, the rabbits receiving similar doses by identical routes nevertheless showed marked variation in their plasma salicylate levels, even though these were determined each morning eight hours after the last injection and just prior to the next injection in all animals.

Many animals became fatally intoxicated within a short period, while other animals gave no evidence of intoxication. Samples of blood were obtained with difficulty by cardiac puncture from the animals that received salicylates because of the frequency of cardiac tamponade after this procedure. Therefore blood for prothrombin studies and salicylate levels was removed from the femoral veins under direct exposure in nonanesthetized animals. Since so many animals became fatally intoxicated, it was possible to make frequent determinations in only nineteen rabbits.

Following a single injection of sodium salicylate subcutaneously in rabbits, there was a prompt rise in the plasma level to a peak value within thirty minutes. This level then dropped rapidly during the first three hours and was

often too low to measure after four hours. On the other hand, repeated injections produced a cumulative effect which varied considerably in different animals as reflected in the height of the plasma level eight hours after the previous injection.

The plasma prothrombin times were determined by the one-stage method of Quick.[14] This consists in determining the clotting time of recalcified plasma to which an excess of thromboplastin is added. Commercial thromboplastin* was employed. A curve was constructed from the prothrombin times of various concentrations (100, 75, 50, and 25 per cent) of the plasma of a normal person or rabbit. The dilutions were made with physiologic saline. The prothrombin time of the undiluted plasma of the patients or animals receiving salicylates was applied to the curve constructed from the control plasma (rabbit or human), the value of the unknown being expressed as per cent of the normal plasma dilution. A new control curve was prepared each time prothrombin measurements were made. It was not possible to apply human and rabbit prothrombin times interchangeably to the same control curve. The prothrombin times of undiluted and diluted rabbit plasma are much shorter than are those of human controls. Many curves were constructed from the plasma of the same human control and were remarkably constant from day to day. The commercial thromboplastin did not have maximum potency and the slope of the curves in the range 100 to 50 per cent of normal approximated that observed by Souter and Kark,[15] who indicated the advantages of using a relatively weak thromboplastin. With a more potent preparation of thromboplastin, little or no difference in prothrombin time is observed in the 100, 75, and 50 per cent saline dilutions of control plasma.

Link and associates[6, 16] advocate determining the prothrombin time of a 12.5 per cent saline dilution of the unknown plasma. This method is particularly valuable in detecting slight deviations from normal, since at this dilution a slight variation in prothrombin content alters the time interval considerably. These investigators use a potent preparation of thromboplastin, and, in their hands, the method apparently is reliable even with great reduction in plasma prothrombin content. However, with the available commercial thromboplastin, the normal prothrombin time of a 12.5 per cent saline dilution is often as long as 100 seconds. Marked reduction in prothrombin content greatly lengthens this time, often giving an end point which is difficult to read. Butt and co-workers[12] experienced the same difficulty with the 12.5 per cent dilutions in patients receiving sodium salicylate.

Plasma salicylate levels were determined by the method of Brodie, Udenfriend, and Coburn.[17] Values for plasma salicylate are expressed as micrograms of salicylic acid per cubic centimeter of plasma. Dividing this value by 10 gives the concentration in milligrams per 100 c.c. Menadione was the type of synthetic vitamin K employed. It is dispensed as an aqueous solution in 60 mg. ampules. This was administered intravenously and intramuscularly to human beings and intramuscularly to rabbits.

*Winthrop Chemical Co., Inc., New York, N. Y.

RESULTS

The severity of the hypoprothrombinemia of the plasma of patients receiving sodium salicylate usually was proportional to the level of salicylate in the plasma, increasing with higher plasma salicylate levels (Table I, Figs. 1 and 2). In a given individual, this relationship between plasma salicylate level and degree of hypoprothrombinemia was striking. Different individuals, however, showed some variation in the readiness with which the prothrombin level was reduced in association with a rise in the height of the salicylate level. Usually no significant hypoprothrombinemia (below 40 per cent) occurred until the plasma salicylate level exceeded 600 micrograms per cubic centimeter.

In rabbits, as well as in man, there was a definite correlation between the severity of hypoprothrombinemia and the height of the plasma salicylate level (Table II, Fig. 3). When the salicylate level of the plasma was low, there was no reduction in the prothrombin content although the same dose of sodium salicylate administered to other animals frequently produced high levels and hypoprothrombinemia. One rabbit in this study was given sodium salicylate in the usual dosage (0.9 Gm. per kilogram per twenty-four hours subcutaneously) for a period of thirty days, and yet no reduction in the prothrombin content of the plasma occurred. The plasma salicylate level remained low at all times.

Four patients were given menadione during the course of salicylate therapy. In three individuals, doses as large as 60 mg. daily administered intravenously failed to alter the existent hypoprothrombinemia (Figs. 1 and 2). In one case, 120 mg. of menadione injected intravenously on one day followed by 180 mg. by the same route on the next day restored the prothrombin content to normal for a short period (Fig. 2, Case V. W.). A single intravenous infusion of 500 mg. of cevitamic acid which was given to two individuals with hypoprothrombinemia did not have any effect.

Menadione was administered intramuscularly to three rabbits in doses of 10 mg. daily. In each animal, the vitamin K administration was begun on the day that salicylate therapy was instituted. These animals (Fig. 3) developed high plasma salicylate levels and hypoprothrombinemia occurred while repeated injections of vitamin K were being continued.

In spite of the frequent occurrence of marked prolongation of the prothrombin time of the plasma of patients receiving salicylate therapy, there was evidence of bleeding in only one instance. This patient, a healthy adult, had bleeding from the nose and gums at a time when there was marked hypoprothrombinemia (20 per cent of control curve) and severe salicylate intoxication. In another case, with fatal salicylate intoxication and equally severe hypoprothrombinemia, autopsy revealed only a few insignificant hemorrhages in the serous membranes. In at least five other patients with severe salicylate intoxication and great reduction in prothrombin content of the plasma, no spontaneous hemorrhages were observed.

We have produced fatal intoxication in over 100 rabbits by repeated injections of large doses of sodium salicylate. Gross autopsy examinations, which were made in all animals, usually disclosed a few small hemorrhages in serous

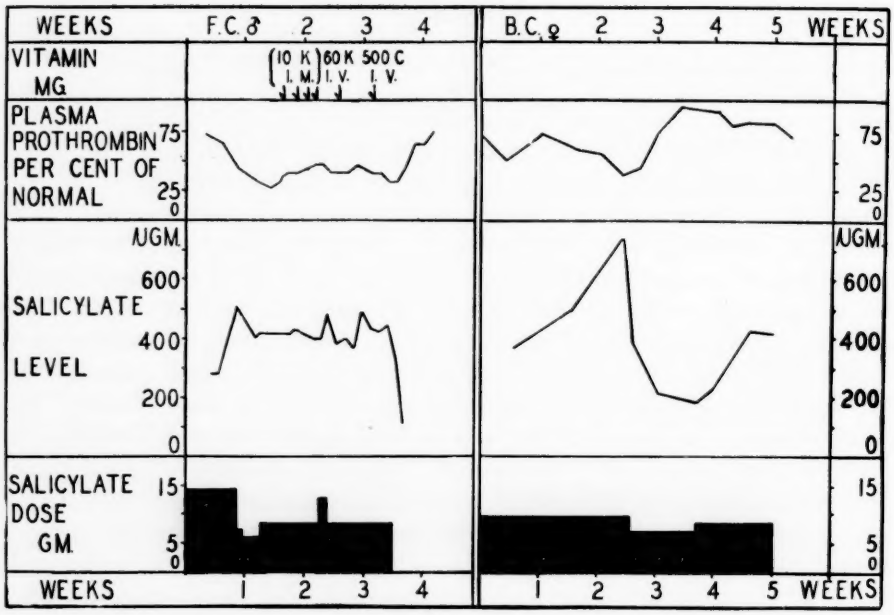

Fig. 1.—The relationship of plasma salicylate level to plasma prothrombin content in two patients. Note the apparent lack of response to vitamin K and vitamin C in the first case.

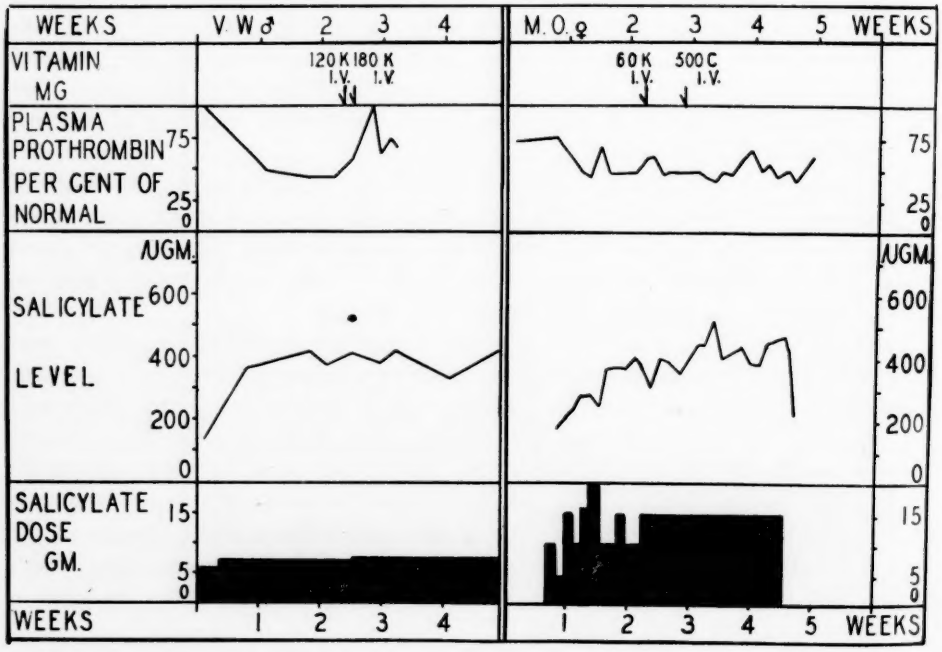

Fig. 2.—The relationship of plasma salicylate level to plasma prothrombin content in two patients. Note the apparent temporary effect of large doses (from 120 to 180 mg.) of vitamin K in the first case and the lack of response to vitamin C and vitamin K in the second case.

TABLE I. RELATIONSHIP OF PLASMA PROTHROMBIN CONTENT TO THE PLASMA SALICYLATE LEVEL IN MAN

	PLASMA PROTHROMBIN CONTENT		
	BELOW 40%	FROM 40-60%	ABOVE 60%
Number of cases	5	14	5
Mean salicylate level (μg./c.c.)	644	424	296
Range (μg./c.c.)	405-810	264-840	260-350

TABLE II. RELATIONSHIP OF PLASMA PROTHROMBIN CONTENT TO THE PLASMA SALICYLATE LEVEL IN RABBITS

	PLASMA PROTHROMBIN CONTENT		
	BELOW 40%	FROM 40-75%	ABOVE 75%
Number of cases	6	9	4
Mean salicylate level (μg./c.c.)	477	337	140
Range (μg./c.c.)	300-640	90-568	20-280

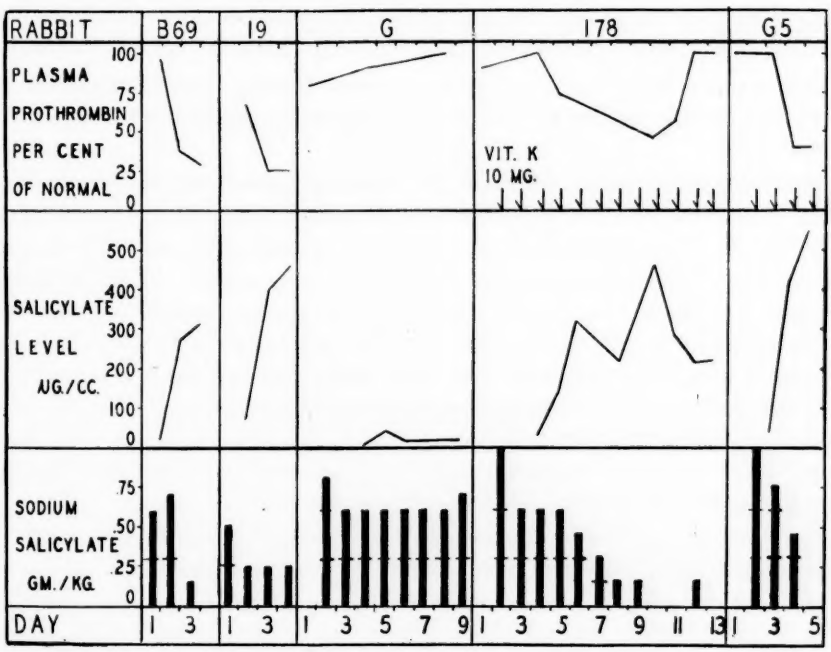

Fig. 3.—The relationship of plasma prothrombin content to plasma salicylate level in five rabbits. Note the apparent failure of vitamin K to prevent hypoprothrombinemia in the last two animals.

membranes but never any severe hemorrhage which might have been responsible for the death of the animals (the central nervous system was not examined).

Some in vitro studies were made to determine the influence of sodium salicylate upon the prothrombin content of the plasma. The concentration of sodium salicylate (added as dry powder) varied from 250 to 1,000 micrograms per cubic centimeter in various experiments. Prothrombin times were determined immediately; after twenty-four hours' incubation at 37° C.; after forty-eight and ninety-six hours at 4° C.; and after 120 hours at room temperature,

using sterile technique. At the temperature of 4° C., the prothrombin content of both the control plasmas and the plasmas to which salicylates had been added were little affected, even after from two to four days' standing. With the higher temperatures, a progressive reduction of prothrombin content of equal degree occurred in both control plasmas and in those containing salicylates. This action was more pronounced at 37° C., particularly after prolonged incubation. A similar destruction of prothrombin in normal plasmas permitted to stand at room temperature has been reported by Taylor and co-workers.[18] The failure to demonstrate an in vitro action of salicylate in concentrations attainable in vivo has also been observed by Link and co-workers.[3]

COMMENT

These studies indicate that the severity of hypoprothrombinemia in patients and experimental animals may be correlated with the height of the plasma salicylate level, the degree of hypoprothrombinemia increasing with high salicylate levels. The plasma salicylate levels in a given individual may not be predicted from the dose employed since there is great individual variation in the dosage necessary to obtain comparable plasma levels in different persons.

Butt and co-workers[12] likewise noted the hypoprothrombinemia associated with salicylate medication in patients with rheumatic fever but could find no correlation between plasma salicylate level and degree of reduction in plasma prothrombin. They expressed the opinion that the likelihood of development of severe hypoprothrombinemia and spontaneous hemorrhage in association with salicylate medication was remote. We are in accord with this opinion. Recently in a preliminary report, Coombs, Higley, and Warren[19] observed that the hypoprothrombinemia occurring with salicylate therapy is in direct proportion to the plasma salicylate level and that the severity of hypoprothrombinemia is of minor degree until the plasma salicylate level reaches 600 micrograms per cubic centimeter. This confirms our observations.

Previous reports have emphasized the value of vitamin K in preventing the development of hypoprothrombinemia as well as in hastening the restoration of the prothrombin content when salicylates have been discontinued.[4, 5, 13] In the present work, limited observations in rabbits indicate that when the plasma salicylate level is high, hypoprothrombinemia may occur in spite of continued administration of vitamin K. In man, we did not begin vitamin K medication at the time of initiating salicylate therapy; however, moderately large doses of vitamin K (from 60 to 120 mg. intravenously) after the hypoprothrombinemia had developed had little or no effect while salicylate therapy was being continued.

The mode of action of salicylates upon the prothrombin content of the plasma would seem to be via the liver. It is a well-established fact that vitamin K is necessary for synthesis of prothrombin by the liver. Animals fed diets low in vitamin K[3] and patients with cirrhosis of the liver[5] have been found to be more susceptible to the hypoprothrombinemic effect of salicylates. In the patients and rabbits studied in the present work, there was no reason

to suspect a dietary lack of vitamin K or the presence of antecedent damage to the liver.

Since salicylates, dicumarol, and vitamin K contain salicyl groups in their molecular structures, it seems possible that, although sufficient vitamin K is present, its utilization may be blocked by salicylates or by dicumarol. Massive doses of vitamin K may overcome this interference, perhaps by a mass action effect. The hypoprothrombinemia produced by dicumarol in patients usually may be overcome by the administration of very large doses of vitamin K.[20, 21]

The lowering of the prothrombin content may be the result of the influence of salicylates on certain enzyme systems. Serious liver impairment does not appear to be a frequent finding during salicylate medication, especially as evidenced by the usual liver function tests.[12] However, ketonuria may occur if salicylate intoxication is severe. Moreover, in vitro studies have demonstrated alterations of certain metabolic activities of tissue slices in the presence of salicylates.[22-25] The rapid restoration of the prothrombin content of the plasma after the discontinuance of salicylate therapy does not suggest that any permanent liver damage occurs.

SUMMARY

A correlation between the height of the level of salicylate in the plasma and the degree of hypoprothrombinemia was established as a result of studies on twenty-four patients and nineteen rabbits receiving large doses of sodium salicylate.

Moderately large doses of vitamin K administered to rabbits throughout the period of salicylate medication and to man during the course of salicylate medication did not appear to have any effect in regard to the development or persistence of hypoprothrombinemia while salicylate therapy was continued.

The prolonged prothrombin time does not appear to be of clinical significance since hemorrhagic phenomena in intoxicated animals and man are infrequent and, when present, usually do not appear to be a factor in causing death from salicylate intoxication.

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A CRITICAL ANALYSIS OF THE VALUE OF THE ADDITION OF A AND B GROUP-SPECIFIC SUBSTANCES TO GROUP O BLOOD FOR USE AS UNIVERSAL DONOR BLOOD

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IN A RECENT paper[1] it was demonstrated that transfusions of group O blood with an isoagglutinin titer of 400 units or higher (by the tube-centrifuge method of titration) to recipients of other blood groups resulted in hemolytic reactions of varying severity. On the basis of this work, only group O blood with an isoagglutinin titer of 200 units or less was recommended as safe for routine use as universal donor blood. Many investigations have been carried out (Witebsky and associates,[2] Klendshoj and co-workers,[3] and Wiener and associates[4]) on the use of soluble A and B group-specific substances for addition to group O blood to reduce its isoagglutinin titers to safe levels. The ability of these substances to reduce the titer of anti-A and anti-B agglutinins in vitro has been established. Klendshoj and associates[3] have reported clinical studies in a large series of patients on the effect of transfusions of *random* group O bloods to which solutions of group substances had been added. However, no studies have been made of the effects of the transfusion of *selected* group O bloods known to have a high titer of isoagglutinins which had been treated with A and B substances and then given to recipients of other blood groups.

Accordingly, this investigation was undertaken with the following problems in mind: (1) to determine the amount of blood, with a high titer of isoagglutinins, necessary to cause a hemolytic reaction in a recipient of a different blood group; (2) to study the in vitro effects of group-specific substances A and B on plasma with a high isoagglutinin titer*; (3) to determine the effects of the transfusion of blood with a high isoagglutinin titer, after modification with A and B group substances, to recipients of incompatible blood groups.

MATERIALS AND METHODS

For this investigation, blood plasma was used instead of whole blood because the antibodies, whose effects were being studied, are present only in the plasma, so that one could be sure that any hemolysis which occurred was due to destruction of the red cells of the recipient.

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*The group-specific substances were generously supplied by Dr. E. J. Teeter, of Eli Lilly and Co., Indianapolis, Ind.

Group B plasma, having a very high titer of anti-A agglutinins, was obtained* from a blood donor who had been immunized by an injection of soluble group-specific substances. The titer of the anti-A agglutinins for A_2 cells, by the well-slide method, reached as high as 2,500 units (equivalent to 15,000 units by the tube-centrifuge technique) but had fallen to a titer of 500 units (2,500 units by the tube technique) by the time the plasma was collected from the donor some three months after the injection of the group substances. One thousand cubic centimeters of this plasma were obtained at one time by withdrawing 1,800 c.c. of blood from the donor and simultaneously replacing it with an equal volume of group B bank blood.

This plasma, both in the untreated form and with A and B group substances added in the proportion of 10 c.c. of W. Tebsky's solution to 250 c.c. of plasma, was given in varying doses to eight volunteer recipients recruited through the cooperation of the Colorado State Penitentiary.† Twelve 250 c.c. bottles of group O plasma with high anti-A agglutinin titers‡ were administered, after neutralization with 10 c.c. of A and B substances, to twelve other volunteers.

Each volunteer recipient was given a thorough physical examination. Only healthy men were used. The recipient's blood group was determined and he was classified as a secretor or a nonsecretor, according to the technique of Wiener.[5] Volunteers of subgroups A_1 and A_2 alone were transfused. The plasma was titrated for its isoagglutinin content against the red cells of the prospective recipient. Except where otherwise indicated, all titrations were performed by the tube-centrifuge technique. In those cases in which the A and B soluble substances were added, titrations against the recipient's red cells were performed before and after neutralization. Twenty-four hours prior to each transfusion, the hemoglobin concentration (using the Fisher hemometer), red blood cell count, hematocrit, serum bilirubin, and urine urobilinogen values of each recipient were ascertained.§ These tests were repeated one hour after the end of the transfusion and every twenty-four hours thereafter for seven days. Immediately at the termination of the infusion, the recipient's blood was examined for evidence of spontaneous agglutination. The technique for determining this phenomenom and its significance have been discussed previously.[1] All recipients were alkalinized with sodium bicarbonate before the plasma was given.

RESULTS

Our findings are summarized in Table I. The data for the third, fifth, and sixth days are not given for the sake of brevity and because they showed no significant deviation from the days shown.

As noted in Table I, as little as 25 c.c. of the untreated group B plasma administered to a group A_1 recipient (Case 1) caused a frank hemolytic reaction, as manifested by chill, fever, pain in the lumbar region, hemoglobinuria,

*We gratefully acknowledge the aid given us by Dr. Lester J. Unger, of the New York Post-Graduate Hospital Blood Bank.

†We desire to express our deep appreciation for the cooperation of Warden Roy Best and the volunteers of the Colorado State Penitentiary, Canon City, Colo.

‡Furnished to us by the Department of Surgical Physiology at the Army Medical School, Washington, D. C., through the courtesy of Major John McGraw, Medical Corps, Army of the United States.

§Colonel Hugh S. Mahon, Medical Corps, United States Army, Chief of Laboratory Service, Fitzsimons General Hospital, Denver, Colo., generously provided the services of his laboratory.

and increased bilirubinemia, together with evidence of spontaneous agglutination. Fifty cubic centimeters caused an even more pronounced reaction in another group A_1 recipient (Case 2). In a volunteer of group A_2 (Case 6), 25 c.c. of this plasma caused no obvious reaction, but 50 c.c. (Case 7) gave rise to convincing clinical evidence of hemolysis of the recipient's cells. This confirms our previous work on this subject and demonstrates the small quantity of blood with a high isoagglutinin content that is sufficient to cause a hemolytic reaction if given to recipients of an incompatible blood group. On the other hand, when as much as 250 c.c. of this group B plasma were administered, after addition of 10 c.c. of the Witebsky group substances, to two volunteers (one group A_1, the other A_2), no manifest signs of hemolysis resulted. In one instance only was evidence of spontaneous agglutination observed, but this patient (Case 5) showed no other convincing findings indicating hemolysis.

Twelve additional A_1 recipients were then given transfusions of the group O plasmas containing a high titer of anti-A agglutinins which had been modified by the addition of A and B group specific-substances in the proportion of 10 c.c. of the group substances to 250 c.c. of plasma. As shown in Table I, the ability of these substances to reduce the in vitro isoagglutinin titers to low levels is established beyond question. The highest titer among the plasmas used was 4,800 units by the tube method. The titer of this plasma was reduced to 100 units by the group substances. In one case in which the original titer was 500 units the presence of anti-A agglutinins could no longer be detected after the addition of the group-specific substances.

No clinical reaction was noted among the individuals receiving "neutralized" plasma except for one case of urticaria which has no bearing on this investigation. No post-transfusion hemoglobinuria was observed, and in only six of the twelve recipients was there a significant increase in bilirubinemia. In the other six recipients, while no marked rise in bilirubinemia occurred, a slight fall in the hemoglobin and red blood cell and hematocrit values suggested a small amount of red cell destruction. However, this could also be explained by the hemodilution resulting from the plasma infusion. In five of the recipients, signs of hemolysis were somewhat delayed, appearing as late as the fourth and fifth post-transfusion day. This might be explained by postulating that the combination of agglutinins and the group substances was a loose one, with a gradual release of the agglutinins. Nevertheless, it is felt that in all these recipients the actual amount of red cell destruction was clinically insignificant. This is predicated on the close observation of the recipients, on the fact that no clinical reactions occurred, and on the fact that in all recipients the evidence of hemolysis was transitory and because the small number of recipient's red cells destroyed probably did not exceed the equivalent of from 25 to 50 c.c. of blood. Therefore, it may be stated that this study has demonstrated the ability of A and B group-specific substances to inhibit the hemolytic action of the transfusion of group O blood with a high agglutinin titer to recipients of other blood groups.

As has already been mentioned, every recipient's saliva was tested for the presence or absence of group-specific substance A, and each patient was classified

TABLE I

CASE*	RECIPIENT'S BLOOD GROUP	SALIVA TEST	AMOUNT TRANSFUSED (C.C.)	AGGLUTININ TITER OF PLASMA AGAINST RECIPIENT'S CELLS *before* ADDITION OF A AND B SUBSTANCE	AGGLUTININ TITER OF PLASMA AGAINST RECIPIENT'S CELLS *after* ADDITION OF A AND B SUBSTANCE	TWENTY-FOUR HOURS BEFORE TRANSFUSION					ONE HOUR AFTER TRANSFUSION						TWENTY-FOUR HOURS AFTER TRANSFUSION		
						HEMOGLOBIN (GM.)	R.B.C.	HEMATOCRIT	BILIRUBIN (MG./100 C.C.)	UROBILINOGEN	SPONTANEOUS AGGLUT.	HB. (GM.)	R.B.C.	HEMATOCRIT	BILIRUBIN (MG./100 C.C.)	UROBILINOGEN	HEMOGLOBIN (GM.)	R.B.C.	HEMATOCRIT
1†	A_1	Sec.	25	1-2400		15	4,680	44.5	1.10	Neg.	Pos.	15.4	4,850	46	2.10	Neg.	14	4,800	43
2†	A_1	Sec.	50	1-2400		17	5,810	47.5	0.60	Neg.	Pos.	16	5,600	46.5	2.38	Neg.	16	5,570	48
3	A_1	Sec.	25	1-2400	1-40	16	5,950	47.5	0.42	Neg.	Neg.	15	5,300	48	1.00	Neg.	14.7	5,730	49
4	A_1	Sec.	50	1-4800	1-100	14.8	5,500	47.5	0.38	Neg.	Neg.	15	5,250	47	1.18	Neg.	15	5,190	47
5	A_1	Sec.	250	1-4000	1-32	15	5,470	48	0.59	Neg.	Pos.	14.2	4,690	42	0.82	Neg.	14	4,850	41
6†	A_2	Sec.	25	1-2000		14	5,290	46	0.80	Neg.	Pos.	13.3	4,960	41.5	1.20	Neg.	14.8	4,890	45
7†	A_2	Nonsec.	50	1-1500		14.8	5,490	44	0.87	Neg.	Pos.	14.8	5,650	45	1.40	Neg.	14.2	5,810	46
8	A_2	Nonsec.	250	1-1000	1-4	13.8	4,980	45	0.40	Neg.	Neg.	13.5	4,660	42	0.87	Neg.	13.8	4,820	43.5
9	A_1	Nonsec.	250	1-1200	1-24	16	5,400	45	0.50	Neg.	Neg.	13.5	5,170	42	1.10	Neg.	13.5	4,900	43
10	A_1	Sec.	250	1-800	1-12	16	5,600	47.5	1.05	Neg.	Neg.	16	5,340	42	0.70	Neg.	15.5	5,250	44
11	A_1	Sec.	250	1-1200	1-8	16.5	6,010	51.5	0.90	Neg.	Neg.	15.8	5,650	48	0.50	Neg.	15.8	5,500	51
12	A_1	Sec.	250	1-1000	1-8	13.9	4,750	43	0.30	Neg.	Neg.	15.8	4,750	44.5	0.90	Neg.	16	5,310	46
13	A_1	Sec.	250	1-600	1-4	14.4	5,240	46	0.46	Neg.	Neg.	12.5	4,810	43	0.65	Neg.	14.2	5,980	49.5
14	A_1	Sec.	250	1-1000	1-8	15.5	5,850	50	0.80	Neg.	Neg.	16	5,450	45	0.87	Neg.	13.6	5,320	47
15	A_1	Sec.	250	1-1000	1-4	15	5,800	46	0.70	Neg.	Neg.	14.2	5,530	44	0.87	Neg.	16	5,490	49
16	A_1	Nonsec.	250	1-1000	1-8	14.6	5,340	45.5	0.45	Neg.	Neg.	14	4,900	42	0.59	Neg.	14	5,280	44
17	A_1	Sec.	250	1-2000	1-8	15.4	5,920	44.5	0.40	Neg.	Neg.	12.8	4,860	40	0.59	Neg.	13.2	4,960	39
18	A_1	Sec.	250	1-1000	1-4	13.8	5,180	43	0.70	Neg.	Neg.	14.2	4,950	43	1.07	Neg.	15.5	5,270	43.5
19	A_1	Sec.	250	1-1500	1-4	15.2	6,100	46	0.59	Neg.	Neg.	14.4	5,590	43	0.70	Neg.	16	5,630	47
20	A_1	Sec.	250	1-500	None detected	15.4	5,620	47.5	0.30	Neg.	Neg.	15	5,370	44.5	0.40	Neg.	15	5,490	46.5

*In Cases 1 to 8, the recipients received group B plasma obtained from one special donor. In Cases 9 to 20 the recipients received Group O plasma obtained from individual donors.

†Received unmodified plasma.

as a secretor or a nonsecretor. Whether the recipients were secretors or nonsecretors had no obvious effect on the results of the transfusion experiments.

During the course of this project, determinations were made of the titer of the anti-B agglutinins in the sera of those group A recipients who had received group O plasma neutralized with A and B substances. The titrations were performed before the transfusion and at weekly intervals thereafter. Only those recipients who had received modified group O plasma were studied because group O plasma itself does not contain A or B group substances. Therefore, any antibody stimulation in those recipients would be due solely to the action of the added A and B group-specific substances. It has been shown previously that such substances do stimulate antibody formation and indeed their injection has been used to produce potent blood grouping serum of a

TABLE I

HOURS FUSION		SECOND DAY AFTER TRANSFUSION					FOURTH DAY AFTER TRANSFUSION					SEVENTH DAY AFTER TRANSFUSION					CLINICAL REACTION
BILIRUBIN (MG./100 C.C.)	UROBILINOGEN	HEMOGLOBIN (GM.)	R.B.C.	HEMATOCRIT	BILIRUBIN (MG./100 C.C.)	UROBILINOGEN	HEMOGLOBIN (GM.)	R.B.C.	HEMATOCRIT	BILIRUBIN (MG./100 C.C.)	UROBILINOGEN	HEMOGLOBIN (GM.)	R.B.C.	HEMATOCRIT	BILIRUBIN (MG./100 C.C.)	UROBILINOGEN	
1.05	Neg.	16.2	4,800	45	0.78	Neg.	14.5	4,870	44.5	0.80	Trace	15	4,900	45	0.45	Trace	Temperature, 99.4° F.; chill; lumbar pains; hemoglobinuria
0.95	Neg.	16.5	5,500	50	0.86	Neg.	15.5	5,790	45	0.88	Trace	16.4	5,830	48	0.70	Neg.	Lumbar pain; hemoglobinuria
0.50	Neg.	14	5,490	46	0.50	Neg.	14.2	6,130	46.5	0.30	Neg.	15.5	5,990	44	0.35	Neg.	None
0.70	Neg.	16	5,530	47	0.70	Neg.	13.8	5,590	46	0.30	Neg.	15	5,710	43.5	0.15	Neg.	None
0.65	Pos.	14.2	4,710	39	0.75	Trace	15.5	5,250	46	0.72	Neg.	14.6	5,430	43	0.65	Neg.	None
1.15	Trace	14	5,100	47	0.90	Pos.	13.8	5,020	43	0.65	Neg.	14	5,480	42	0.72	Neg.	Chill; temperature, 100° F.
0.28	Neg.	13.8	5,690	45	0.70	Neg.	15.8	5,420	45.5	0.72	Neg.	15	5,290	40	0.90	Neg.	Hemoglobinuria; chill
0.72	Trace	15.5	4,990	47	0.50	Trace	15.8	4,690	44.5	0.70	Trace	14.8	5,060	41.5	0.20	Neg.	None
0.45	Neg.	14	5,150	43.5	0.30	Neg.	13.8	5,160	42	0.70	Neg.	13	4,910	45	0.59	Neg.	Urticaria
0.80	Neg.	15	5,550	43.5	0.82	Neg.	15.3	5,420	45	1.60	Neg.	15	5,270	46	1.55	Neg.	None
0.70	Neg.	14.2	5,450	48.5	0.78	Neg.	15	5,060	46.5	0.30	Trace	15.8	5,890	45.5	1.05	Neg.	None
1.35	Neg.	13	4,290	39.5	0.72	Neg.	13.6	4,730	41.5	0.07	Neg.	13.2	4,420	38	0.25	Neg.	None
0.88	Neg.	14.8	5,070	43.5	0.83	Neg.	13.2	4,570	41	0.92	Neg.	13.2	5,220	42	0.54	Neg.	None
0.80	Neg.	13.5	5,410	47	0.78	Neg.	14.3	5,710	47	0.87	Trace	14.8	5,810	47	0.62	Neg.	None
0.40	Neg.	16	5,700	48	0.70	Neg.	15	5,770	47.5	0.42	Neg.	15.2	5,410	49	0.68	Neg.	None
0.50	Neg.	15	5,740	46	0.80	Neg.	13.5	5,080	41	1.30	Neg.	15	5,530	45	0.30	Neg.	None
0.50	Neg.	15.4	5,350	42	0.35	Neg.	14	5,410	44.5	1.05	Neg.	15.5	5,810	44.5	0.90	Neg.	None
0.72	Neg.	15	5,190	45	0.85	Neg.	15.2	4,890	40.5	1.35	Pos.	15.4	5,260	40.5	0.40	Neg.	None
0.68	Trace	15.2	5,480	46.5	0.85	Neg.	16.5	5,670	46	0.70	Neg.	15.2	5,590	45	0.87	Pos.	None
0.90	Neg.	14	5,640	45	1.05	Pos.	14	5,510	45.5	0.80	Pos.	15.3	5,880	47	0.40	Trace	None

high isoagglutinin content.[6, 7] The results of these titrations are presented in Table II.

It is evident that a definite rise in the anti-B agglutinin titer occurred in all twelve recipients. On the average, there was a sixfold increase in the titers. This, in itself, presents no problem in male recipients. However, in a recent report, Wiener[8] has described a case of erythroblastosis fetalis caused by the action of the anti-A isoagglutinins of a group O mother on the group A red cells of a fetus in utero. Such an occurrence is more likely when there is a high titer of isoagglutinins in the maternal circulation. It follows, therefore, that there is a slight but definite danger in stimulating increased isoantibody formation in female patients potentially capable of childbearing. On this basis it may be wise to be more discriminating about transfusing such women with group O

TABLE II. TITRATIONS OF ANTI-B AGGLUTININS IN GROUP A RECIPIENTS OF NEUTRALIZED GROUP O PLASMA

CASE	BEFORE TRANSFUSION	FIRST WEEK	SECOND WEEK	THIRD WEEK	FOURTH WEEK
9	1-10	1-20	1-30	1-30	1-40
10	1-10	1-20	1-40	1-80	1-100
11	1-40	1-80	1-120	1-140	1-180
12	1-40	1-80	1-80	1-80	1-100
13	1-30	1-40	1-60	1-60	1-80
14	1-10	1-40	1-80	1-100	1-140
15	1-20	1-40	1-60	1-80	1-100
16	1-10	1-20	1-40	1-80	1-100
17	1-10	1-20	1-40	1-80	1-80
18	1-120	1-150	1-150	1-180	1-300
19	1-100	1-150	1-180	1-200	1-300
20	1-40	1-40	1-100	1-120	1-180

blood modified by group-specific substances. Certainly, however, in the face of an emergency, there should be no hesitancy in using such blood.

COMMENT

There are two fields in medicine where the use of universal donor group O blood plays an important role. It is of prime importance in military medicine in the treatment of combat casualties, where its efficacy has been proved beyond doubt. It has been demonstrated that group O blood must have a low titer of isoagglutinins (about 200 units by the tube technique) to be safe for use as universal donor blood.[1] Such a low titer will mean discarding about 25 to 50 per cent of group O bloods to be used for this purpose. In the presence of a large demand for universal donor blood, such a waste of material would be unwarranted. Based on the observations made in the present study, it is felt that the routine addition of A and B substances to group O blood would render any group O blood perfectly safe for use as universal donor blood for military purposes.

The other important therapeutic role of universal donor blood is its use for the immediate treatment of emergency cases of hemorrhage in civilian life. Low titer group O blood should be transfused while proper groupings and cross-matching tests are being carried out. As an additional precaution, Rh-negative blood should be used. Further observation and follow-up studies must be made on the subject of the possible significance and effects of isoantibody stimulation in women potentially capable of childbearing. In the meantime, because of the impracticability of having two kinds of universal donor blood, one for men and one for women, we believe that low-titer group O blood destined for use as universal donor blood in civilian hospitals should be selected according to the technique outlined in our previous paper,[1] without the addition of A and B substances.

CONCLUSIONS

1. As little as 25 c.c. of a group B plasma containing a high titer of isoagglutinins caused a hemolytic reaction when administered to a group A recipient.

2. A and B group-specific substances will reduce a high isoagglutinin titer to low levels.

3. Experiments on the administration of plasma with high titers of anti-A agglutinins to group A recipients present definite evidence that the addition of group-specific substances A and B is a safe and reliable method for preparing all group O blood for use as universal donor blood for military purposes.

4. A and B substances are capable of antibody stimulation even when in combination with the anti-A and anti-B agglutinins of group O blood. This calls for some caution in administering such material to women potentially capable of childbearing.

5. For use as universal donor blood in civilian hospitals, Rh-negative group O blood, selected for its low natural isoagglutinin titer, is somewhat preferable to random group O blood to which group substances have been added to reduce the isoagglutinin titer.

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UREA AS AN ADJUNCT TO SULFONAMIDE THERAPY

Report of Its Successful Use in a Case of Subacute Bacterial Endocarditis

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THE renal complications from the use of sulfadiazine are well known and have been adequately described in recent medical literature. When the drug is given in massive doses the possibility of obstructive and toxic damage to the kidney is greater. The administration of alkali has been helpful in preventing or reducing these untoward effects. Urea, too, can be used for this purpose and, in addition, may add to the antibacterial action of the sulfonamide.

Urea or carbamide is a white, colorless, odorless, crystalline substance, highly diffusible, dissolving readily in water, 1 Gm. in 1.1 c.c.[1] Aqueous solutions are neutral and, on heating, break down with the liberation of ammonia and carbon dioxide and, therefore, are not to be sterilized by heating. Urea is marketed in chemically pure form and also as urea reagent,[1] which contains impurities. Obviously the former and not the latter is used for intravenous therapy. On local application it is nontoxic, is fairly innocuous to human tissues, and can be sprinkled directly on wounds.[2]

Long ago (1902) Ramsden[3] showed that it prevented putrefaction and in as dilute solution as 2 per cent had a marked effect in promoting digestion by proteolytic enzymes. Foulger and Foshay[2] showed that some enzymatic actions may be retarded even by low concentrations.

The first detailed study of urea as a bactericide was made by Peju and Rajat in 1906.[4] In the same year, Wilson[5] noted that growth of *Escherichia coli* could be prevented by 8 per cent urea—from 1.5 to 3.5 per cent caused pleomorphism of these organisms.

Urea was used for its direct action in World War I by Symmers and Kirk[6] to débride contaminated war wounds chemically. They applied (100 per cent) aqueous solutions and also used the solid substance. Kirk had a favorable experience with its clinical use over a period of four years. Holder and Mackay[7] in 1937 found it of value in the treatment of infected wounds. In 1942 these authors[8] demonstrated its use together with sulfanilamide in the treatment of infected wounds. They believed the carbamide (urea) action rids the wound of necrotic tissue and similarly of sulfonamide inhibitors, allowing the sulfonamide to exert a maximum bacteriostatic action. They also stated that urea markedly enhanced the solubility of sulfonamides and thus increased their action. Their experience covered a five-year experimental period. This type of inactivation of sulfonamide inhibitors was also described with the use of azochloramide[9] and urethane.[10]

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During 1942 studies in the therapeutic application of urea in infections were reported by Tsuchiya, Tenenberg, Clark and Strakosch.[11] They found that urea (1) was nontoxic, a strong peptizing agent with marked solvent action for necrotic tissue, pus, and debris; (2) chemically débrided contaminated wounds and mechanically removed inhibitors; (3) lysed bacteria, deodorized foul-smelling wounds, and rendered sulfonamides more soluble. They particularly showed urea to neutralize methionine and remove sulfonamide fastness. The bacteria used were *Esch. coli* and a staphylococcus which was obtained from a human abscess and made "fast."

Curtis and Sobin, Sobin, and Sobin, Aronberg, and Rolneck[12] (1941-1943) called attention to the sulfonamide-solvent effect of urea and showed that urea might prevent the formation of renal calculi by sodium acetyl sulfapyridine in rats.

Thus far the work had been on the action of urea locally in wounds, and usually in high concentration, in vitro, and in the kidney of rats.

In 1943, when we contemplated treating a patient with subacute bacterial endocarditis with massive doses of sulfadiazine, it was suggested to us by Dick[13] that urea be used with it. The following is a report on a patient with subacute bacterial endocarditis in whom this method of the use of the sulfadiazine and urea was employed and in whom recovery promptly followed.*

REPORT OF CASE

History.—R. K., a native-born white girl aged 18 years, single and attending trade school, was admitted to the medical service of Beth Israel Hospital Jan. 6, 1943. She was an only child and was told she was born with a weak heart but was not a blue baby. Since the age of 6 years she attended a cardiac clinic about twice a year. During her childhood she played games such as ball and jumping rope without undue difficulty. Her physical activity was not limited until three years before admission to the hospital and then only on the advice of a physician. There was no dyspnea, edema, or palpitation.

About two months prior to admission to the hospital she began to cough, lose weight, and feel ill. This continued for a few weeks, at which time she was seen by a physician who told her she had fever and should remain in bed. Anorexia developed and her temperature rose to 101° F. After several days in bed she felt better and returned to school.

One day before admission to the hospital she became more feverish, visited her physician again, and was advised to seek hospitalization. She had lost thirteen pounds during the two months' illness, felt tired and weak all the time, and had missed her last two menstrual periods.

Examination.—The patient was 5 feet, 3 inches tall, weighed 122 pounds, sthenic habitus, appeared acutely ill and quite pale. She was coughing and complained of some pain over the left lower chest and left upper abdomen, aggravated by deep breathing and by coughing. The temperature was 103.2° F.; pulse rate, 102; and respiratory rate, 24. There was slight dyspnea and moderate cyanosis of the nail beds. Petechiae were not found in the conjunctivae or fundi nor on the body or extremities.

The pharynx was slightly congested; teeth were in good repair; the cervical lymph nodes were not palpable. The main features of interest were found on examination of the heart and lungs.

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*In the September, 1944, issue of the JOURNAL, Schnitker and Lenhoff[14] reported the use of urea and sulfonamide by mouth in the treatment of sulfonamide-resistant gonorrhea.

In the Feb. 10, 1945, issue of the Lancet Ecker[15] reported the use of urea and sulfadiazine in the treatment of a patient with *B. coli* meningitis.

The heart was not enlarged to percussion; the apical impulse was of moderate force and within the midclavicular line. The first sound at the apex was of normal quality and intensity. There was a loud rough blowing systolic murmur heard over the whole precordium, maximum at the third intercostal space and fourth rib just left of the sternum. The murmur was transmitted to the vessels of the neck and to the interscapular area. The heart sounds at the base were normal. P_2 was slightly louder than A_2 but not accentuated. A systolic thrill was felt over the area where the murmur was maximum. Radial pulses were forceful, equal, and regular. Blood pressure was 134/74, subsequently 116/70 left, 120/70 right. The lungs were clear except for a small area of dullness at the left base. The breath sounds over this area were diminished and had a bronchovesicular quality. The spleen was not palpable. There was no clubbing of the fingers and no peripheral edema.

Laboratory Data.—A teleroentgenogram and cardiac fluoroscopy revealed slight cardiac enlargement to the left, presumed to be due to hypertrophy of the left ventricle. There was moderate prominence of the pulmonary conus. An angiocardiogram was interpreted as indicating no enlargement of the chambers or large vessels. There was persistent visualization of the right ventricle most probably indicating an interventricular septal defect with a left to right shunt. The roentgenogram revealed a small shadow in the left lower lobe just outside the cardiac shadow thought to be due to consolidation of the lung.

The four-lead electrocardiogram was normal. There was no deviation of the electrical axis.

An ether test for right to left shunt of the circulation was negative. The maximum specific gravity of the casual specimens of urine was 1024. Tests for albumin and sugar were negative. Occasionally several red and white blood cells were seen per high-power field. Rare hyaline and granular casts were found.

The blood contained 3,500,000 red cells per cubic millimeter and 9.1 Gm. of hemoglobin per 100 c.c. There were 19,500 white cells, of which 82 per cent were polymorphonuclear leucocytes, 17 per cent small lymphocytes, and 1 per cent mononuclear cells. The erythrocyte sedimentation rate (Westergren) was 82 mm. in forty-five minutes.

The Wassermann, Kline, and Kahn tests were negative. A blood culture taken the day after admission failed to reveal any organisms.

The diagnoses were congenital heart disease, interventicular septal defect with left to right shunt, no cardiac failure, subacute bacterial endocarditis, and pneumonia or infarction of the left lower lobe.

Chemotherapy was started with sulfadiazine orally, 2 Gm. for the first dose and then 1 Gm. every four hours, six times a day. After 16 Gm. had been given, during a period of three days, the temperature, which was sustained between 102 and 104° F. during these three days, somewhat suddenly dropped to below 100° F. and then remained between 98.6 and 99.4° F. for nine days. A total of 23 Gm. of sulfadiazine had been given during the five days of this therapy; a maximum blood level of 31.0 mg. per cent was reached. On January 20 angiocardiography was performed, and on that day the temperature rose to 100.8° F. and daily thereafter to about 101° F. until January 26, when it rose to 104.2° F. A blood culture was then taken and revealed 58 colonies of *Streptococcus viridans* per cubic centimeter of blood.

Oral sulfadiazine therapy was reinstituted and a blood level of 15 mg. per cent was soon obtained. Fever continued and after a two-day period of from 100 to 101° F., it rose to 103° F. In conjunction with continued oral sulfadiazine, fever and foreign protein therapy with triple typhoid vaccine intravenously on successive days was started February 9. A shaking chill occurred one-half hour after each injection and lasted about one-half hour. The temperature rose to 105° F. and remained high for approximately four hours. While receiving her seventh treatment the patient developed swelling of the eyelids and a few reddish urticarial lesions on her chest. These disappeared in a few hours. Sulfadiazine was discontinued after the third injection of the typhoid vaccine because of the presence of many red blood cells in the urine. A total of 55 Gm. had been given in thirteen days. After the series of vaccine injections, the temperature dropped to from 98.6 to 100° F. for forty-eight hours. A blood culture on the first day after the last vaccine injection was sterile; a second culture

five days later revealed only 2 colonies of *Streptococcus viridans* in the blood plate but a heavy growth in the glucose broth culture. The fever returned, with the temperature fluctuating between 99 and 102° F. for twelve days. Because of the hematuria with sulfadiazine, sulfanilamide was then given, but this drug produced an increase in the fever and therefore was discontinued; 10 Gm. had been given. On March 1 the blood culture showed 7 colonies of *Streptococcus viridans* per cubic centimeter. An episode of minor pulmonary embolism occurred. Sulfadiazine therapy was reinstituted, using smaller doses (0.5 Gm. four times daily). A transfusion of 500 c.c. of whole blood was given for the anemia (hemoglobin, 66 per cent) and was accompanied by a skin reaction similar to the one after the typhoid injection. A blood culture taken March 15 revealed 32 colonies per cubic centimeter of blood.

At this point massive dose sulfadiazine therapy was considered. Dick[13] suggested the use of 20 Gm. of sodium sulfadiazine with 30 Gm. of urea in 1,500 c.c. of distilled water daily until blood levels of from 50 to 75 mg. per cent of each were obtained. Three series of infusions at weekly intervals were advised. We decided to give this solution intravenously at a rapid rate, completing the infusion within two and two and one-half hours.

During the first infusion the patient developed the same itching and urticaria such as she had had with previous intravenous injections with other substances. They were readily controlled with ½ c.c. of adrenalin intramuscularly. They reappeared later in the course of the infusion but responded to ephedrine, gr. ⅜. After the infusion the patient became nauseated and vomited many times. The blood sulfadiazine level was 37.9 mg. per cent on the next morning. A similar intravenous infusion of sodium sulfadiazine and urea was given twenty-four hours after the first with no reaction but some nausea. The temperature curve during this time showed rises to 101 and 102° F. The following day (March 20) the blood sulfadiazine (free) was 61.9 mg. per cent and the blood urea nitrogen, 42.9 mg.; March 22 the blood sulfadiazine (free) was 33.3 mg. per cent and the urea nitrogen, 75.5 mg. per cent; and March 24 the blood sulfadiazine was 21.4 mg. per cent and urea nitrogen, 90 mg. per cent.

Because of the nausea and vomiting with consequent dehydration, on the third day following the sulfadiazine and urea treatment an intravenous infusion of saline and glucose was given and continued for four days.

The urinary output was not seriously reduced—it did drop to 540 c.c. for twenty-four hours but then increased considerably with the administration of saline and glucose infusions.

A blood culture March 23 was sterile. The temperature dropped to normal for six days following the first sulfadiazine and urea treatment and then gradually rose to 101° F. A blood culture March 29 was also sterile. The second series of infusions was started on March 29 and was identical with the first. The second infusion of this series was given the following day. The sulfadiazine blood and urine levels and the volume output of urine may be seen in Fig. 2. The maximum sulfadiazine blood concentrations reached this time were 62.1 mg. per cent free and 97.8 mg. per cent total. No untoward reaction occurred. The temperature returned to normal April 1, the day after the infusion, at which time the blood culture was sterile. A third series of infusions similar to the previous two were then given. There was a slight urticarial reaction. The blood sulfadiazine level after this third series of infusions rose to 65.4 mg. per cent free and 96 mg. per cent total.

The urine April 9 at a pH of 6.7 contained 361 mg. per cent of free sulfadiazine, 740 mg. per cent of total sulfadiazine, and 490 mg. per cent of urea N. On April 10 the urine at a pH of 6.8 contained 141 mg. per cent of free sulfadiazine, 341 mg. per cent of total sulfadiazine, and 129 mg. per cent of urea N.

The daily urinary output on one day (April 10) dropped from 2,000 to 90 c.c., but the next day rose to 1,500 c.c. On the day of the severe oliguria the urine had from 2 to 3 red blood cells per high-power field, but no sulfadiazine crystals were found.

While red blood cells and white blood cells were frequently reported in the daily urine specimens during the intensive therapy, sulfadiazine crystals were found in only two of the sixteen specimens examined and then only in small numbers.

Following the second series of infusions the temperature returned to normal and remained so from then on until the patient was discharged twenty-nine days later. During

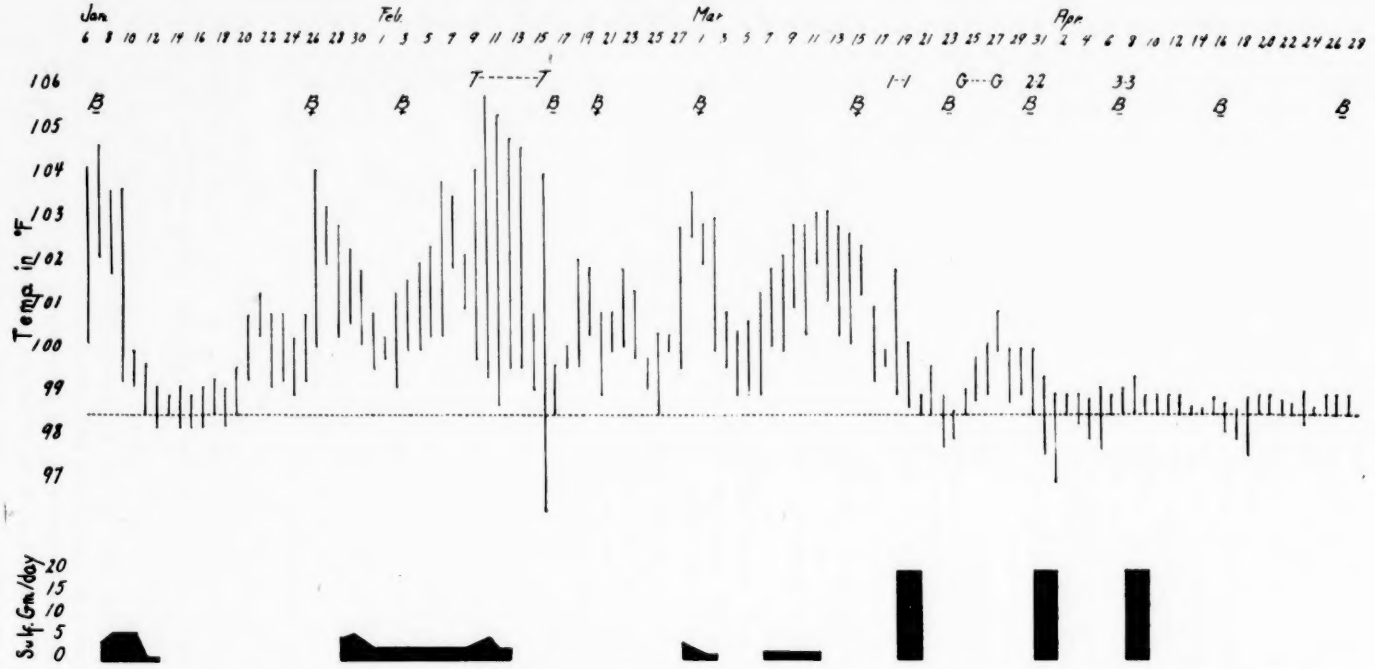

Fig. 1.—Patient's clinical course in the hospital. *B*, Indicates blood culture; *T----T*, daily intravenous typhoid vaccine injections; *G----G*, continuous glucose and saline intravenous infusion. *1-1*, *2-2*, and *3-3*, Intravenous infusions of 20 Gm. sodium sulfadiazine and 30 Gm. urea in first, second, and third series. Hatched areas indicate daily dose of sulfonamide.

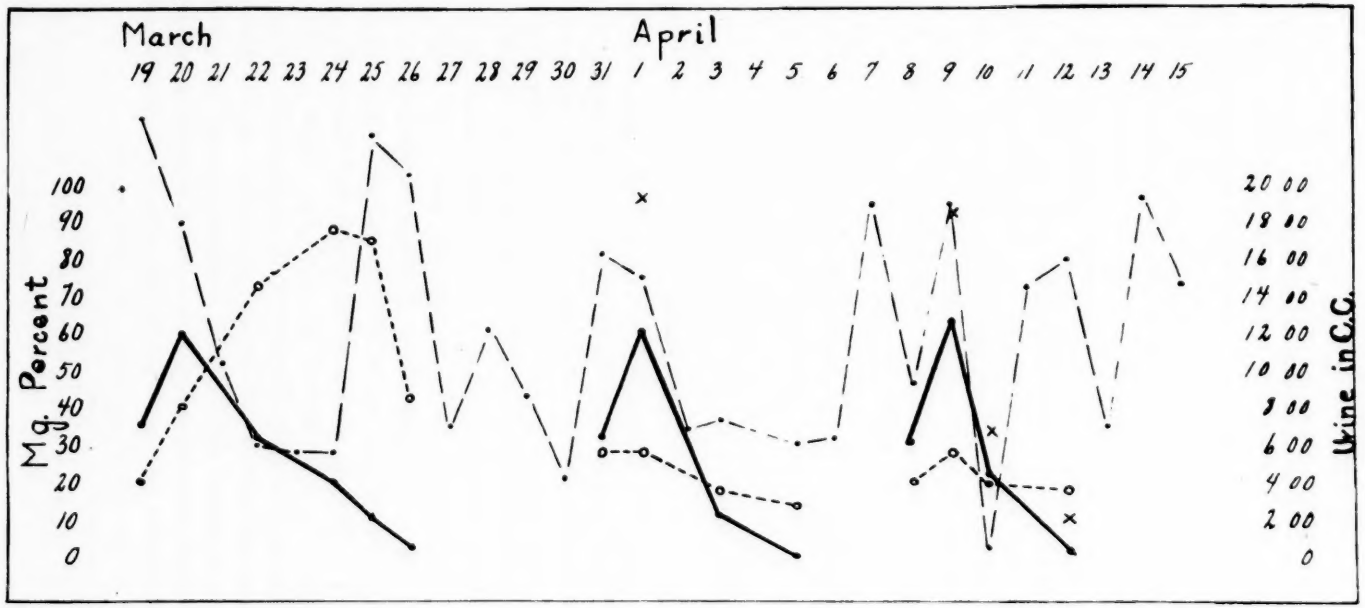

Fig. 2.—Blood sulfadiazine and urea levels and urinary volume output during the period of massive sodium sulfadiazine and urea therapy.

Continuous line, blood sulfadiazine (free); *X*, blood sulfadiazine (total); small dashes, blood urea nitrogen; large dashes, daily urinary volume.

this twenty-nine day period the patient was asymptomatic except for some weakness, which gradually subsided. All blood cultures taken after the administration of the first massive dose of sulfadiazine and urea were negative.

The patient was discharged from the hospital April 29, 1943. After a few months of convalescence at home she went to work and has been working regularly since—a period of over two years. Her weight has increased to 141 pounds, a gain of 32 pounds. She has been feeling well, and at the time of the many examinations during the past two and one-half years, her temperature and pulse rate have been normal. The loud murmur is about the same. The urine has been normal; specific gravity to 1024, negative for albumin, and negative on microscopic examination. The erythrocyte sedimentation rate taken twice was normal. There has been no evidence of recurrence of the infection.

Another patient with subacute bacterial endocarditis received similar treatment with infusions of sodium sulfadiazine and urea but succumbed. Alpha and beta hemolytic streptococci in large numbers were obtained from the blood. Of particular note in this second patient too was the absence of untoward reactions of any kind with the infusions except for cardiac failure during the third series of injections. In vitro determinations revealed the organisms here to be resistant to sulfadiazine, sulfathiazole, sulfapyridine, and sulfanilamide in concentrations up to 8 mg. per cent (pH 7.3). The organisms were also found fast to sulfadiazine in concentrations of 30, 60, and 90 mg. per cent with and without urea. At 116 mg. per cent all the organisms in the culture were destroyed. This high sulfadiazine (free) concentration was found to have been reached in the patient's blood on two occasions.

DISCUSSIONS

During the course of the described treatment with massive doses of sodium sulfadiazine and urea, given intravenously, blood sulfadiazine concentrations were recorded up to 62.1 mg. per cent free and 97.8 mg. per cent total in the first patient and even higher in the second. These are from five to ten times greater than those usually reached and found efficacious in the treatment of pneumonia and other infections, as well as for bacteriostasis in vitro. Blood urea nitrogen concentrations up to 90 mg. per cent were obtained. This is equivalent to 193 mg. of urea or about .2 per cent urea. In the urine, concentrations of total sulfadiazine up to 740 mg. per cent were reached; pH, 6.7. This concentration as well as others obtained are well above the threshold for sulfadiazine crystalluria as indicated by solubility curves (Fox, Jenson, and Mudge[16] and Gilligan, Garb, and Plummer[17]). Nevertheless, there was almost complete absence of sulfadiazine crystals in the urine. In the absence of other known causes, this increased solubility of sulfadiazine may be attributed to the use of urea and so is in accord with the findings of Sobin and co-workers.[12] It has been reported that urea may reduce the viscosity of urine.[18] In this way, too, the solubility of the sulfonamide may be increased.

Little untoward reaction was encountered. Nausea and vomiting were present for a few days after the first infusion. Except for one day of oliguria, no impairment of renal function was found and none is present now, over two years later. In the second patient cardiac failure limited the further use of the infusions.

In the case reported, after other methods of treatment including the use of sulfonamide alone and also combined with typhoid vaccine injections had failed, the use of massive doses of sodium sulfadiazine and urea was promptly followed by recovery of the patient. Because of the promptness of the response and the rarity of spontaneous recovery in subacute bacterial endocarditis,[19] it was felt that the sulfonamide and urea treatment was, in the main, responsible for the recovery.

The treatment has the following possible modes of action:

1. Increased antibacterial (bacteriostatic and bactericidal) power due to higher concentrations of sulfadiazine in the blood.

2. Increased penetration of vegetations in the heart by the higher concentrations of blood sulfadiazine.

3. Increased sulfonamide effect due to the urea: (a) direct potentiation of sulfadiazine[11]; (b) inhibition of antisulfonamide substances—para-aminobenzoic acid (Woods), "P" factor (Green), or other causes of sulfonamide fastness[11]; (c) increased solubility of sulfadiazine in the blood due to the presence of increased concentration of urea.

4. Increased solubility of sulfadiazine in the urine due to the urea,[12] with reduction of crystalluria and consequent renal damage following massive doses of sodium sulfadiazine.

5. Possible direct action of urea on the fibrin protectorate of the bacterial colonies in the endocardial vegetations: (a) promotion of digestion of fibrin by proteolytic enzymes (dilute solutions of urea, 2 per cent, may have such action, Ramsden)[3]; (b) deterrent to coagulation of proteins.[3]

Higher concentrations of urea than those used in the infusions were found to cause local venous thrombosis at the site of injection. The blood urea concentration can be raised by booster oral doses.

It is remarkable that no relapse occurred with such a short duration of treatment.

CONCLUSION

The use of urea as an adjunct in treatment with sodium sulfadiazine is described.

A cure of subacute bacterial endocarditis so treated, with prompt recovery, is reported.

While penicillin is preferable in cases of subacute bacterial endocarditis due to alpha hemolytic streptococcus, in infections where large doses of sulfadiazine are required the use of urea as an adjunct may be of considerable aid. This combined sulfonamide and urea therapy may be desirable in some cases resistant to treatment with penicillin.

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Scientific Program

A CLINICAL METHOD OF DETERMINING THE SPECIFIC RENAL FUNCTIONS OF GLOMERULAR FILTRATION AND MAXIMAL TUBULAR EXCRETION, OR REABSORPTION (AND "EFFECTIVE BLOOD FLOW"), USING A SINGLE INJECTION OF A SINGLE SUBSTANCE

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In a single test, accomplished with little more difficulty than is encountered in performing an intravenous glucose tolerance test plus catheterization, fairly accurate determination of glomerular filtration rate (GFR) and maximal tubular excretion or reabsorption (Tm) can be made. When diodrast (D) or p-amino hippurate (PAH) is employed for the test, determination of plasma clearance (C_D or C_{PAH}) and, therefore, of "effective renal blood flow" is also possible. The procedure for PAH is described as follows:

An amount of p-amino hippurate calculated to yield a plasma concentration of the order of 80 mg. per cent is slowly injected intravenously. Beginning ten minutes later, urine collections are made over suitable periods of time (from five to twenty minutes), and the PAH excretion per minute is determined for each period. A series of blood samples are drawn, and from the curve of declining plasma concentration values are selected corresponding to two and one-half minutes prior to the mid-point of each urinary collection period, as indicative of the average plasma level for that period. (The two and one-half minute correction is applied because of the anachronism due to excretory lag.) The excretion per minute is graphically related to the plasma concentration, using excretions per minute (UV milligrams per minute) as ordinates and plasma concentrations of unbound PAH (PF milligrams per cubic centimeter) as abcissae. A straight line is drawn to fit the points with PF values above 0.15 mg. per cubic centimeter, by inspection or by the method of least squares and is prolonged to the ordinate PF = 0.00.

The slope of this line is the glomerular filtration rate (GFR cubic centimeters per minute $= \frac{\text{milligrams per minute}}{\text{milligrams per cubic centimeter}}$. The UV intercept at PF = 0 is the maximal tubular excretion (Tm_{PAH} = milligrams per minute). A similar

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line applied to points with PF values below 0.05 mg. per cubic centimeter, and running through the origin, has a slope equal to the plasma PAH clearance (C_{PAH}) or "effective renal plasma flow."

While not as accurate as the more standard methods, this procedure is theoretically correct as long as there are no wide fluctuations in renal function or in blood volume during the test. The errors introduced by using a curve of decreasing plasma concentration are fewer than the technical errors of sampling and analysis. The test is more widely adaptable since it eliminates the use of inulin or other substance excreted by filtration only in order to determine filtration. Continuous intravenous infusions are not needed, and ease of analysis of PAH places the procedure within the range of any laboratory which is able to analyze for blood sulfonamide levels.

Comparisons have been made between GFR and simultaneously determined inulin clearance in fourteen cases. The average values of either method were identical. The individual comparisons presented an average difference of ±8 c.c. per minute, or 13 per cent. Each of the values for inulin clearance represents an average of several determinations by the standard method; the average deviations for the fourteen cases were 7 per cent. The average of standard Tm values was also identical to that of simultaneously derived graphic Tm values. Individual comparisons yielded an average difference of ±3 mg. per minute, or 10 per cent. The averaged average deviations of the individual standard Tm values was 6 per cent. Graphic values of C_{PAH} or C_D are, of course, identical to average values calculated in the usual way. The method has been applied to glucose, ascorbic acid, and hippuran figures. The accuracy of GFR determination and of the filtration fraction (the ratio $\frac{\text{GFR}}{\text{renal plasma flow}}$) is reduced where significantly large amounts of material are bound to plasma protein; and the accuracy of Tm determination is reduced in the case of substances where the Tm is small in relation to the analytic sensitivity. An ideal substance would be one whose urinary appearance is governed by glomerular filtration plus a sizable excretory or reabsorptive maximum, is not metabolized, stored in the kidney, or bound to plasma protein, and is nontoxic.

Use of this clinical method enables detection of diminution in the reserves of specific renal functions before a degree of over-all reduction in functional ability is reached which can regularly be demonstrated by present clinical tests.

A COMPARATIVE STUDY OF MERCUHYDRIN AND MERCUPURIN, ORAL AND PARENTERAL

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The diuretic and toxic properties of a new organic mercurial N-(3-methoxy-2-oxymercuripropyl) N-succinylurea, "Mercuhydrin," were studied in 129 patients with edema. The results of this study were compared with those obtained when mercupurin was administered parenterally to fifty-two patients and orally to twenty-five.

All of the patients (206) were confined to bed, received a low-salt diet, and were weighed daily under identical circumstances. Digitalis was given to those requiring it. When weight was stabilized, one of the mercurial drugs was given; if no diuresis resulted, the drug was repeated after three days of ammonium chloride therapy. The amount of weight lost during the twenty-four hours after administration of the diuretic preparation was taken as the measure of the diuretic effect. Blood counts, urinalysis, electrocardiograms, blood nonprotein nitrogen levels, and urea clearance tests were obtained initially and at intervals during the period of observation.

Mercupurin given intravenously in 1 c.c. doses produced an average weight loss of 2.93 pounds; in 2 c.c. doses, 3.24 pounds. Mercuhydrin given intravenously in 1 c.c. doses produced an average weight loss of 2.26 pounds; in 2 c.c. doses, 3.23 pounds. Mercuhydrin was equally as effective when given intramuscularly as when given intravenously. When ammonium chloride was given as preliminary therapy, the weight loss following both mercupurin and mercuhydrin was at least twice that which occurred when the latter drugs were used alone.

Mercupurin administered orally (single dose of five tablets) was less effective than either mercupurin or mercuhydrin given parenterally. Oral mercupurin preceded by ammonium chloride produced a diuresis equivalent to that produced when mercupurin or mercuhydrin was given parenterally without ammonium chloride. Furthermore, failure to diurese occurred more often when mercupurin or mercuhydrin was administered orally.

No significant toxic effects were observed in any of the cases studied. Transient increases in the number of hyaline casts were observed following the parenteral administration of both drugs. This was the only renal change observed.

The new mercurial diuretic mercuhydrin is as effective as mercupurin and can be administered intramuscularly without pain. Oral mercupurin in the doses given is a less effective diuretic than parenteral mercupurin or mercuhydrin.

DISCUSSION

DR. ROBERT M. MOORE, Indianapolis, Ind.—I would like to report one case briefly. A 28-year-old married woman, with one child, had rheumatic heart disease when she was 8 years old. She was seen with a very severe congestive heart failure and was placed on mercupurin. She has now received 148 injections intravenously, which is apparently the one thing that is saving her life. She has an enlarged liver all of the time. I report this case only because over a long period of time she has had this medication and to date there has been no evidence of renal damage.

THE EFFECT OF THEOPHYLLINE AMINOISOBUTANOL IN THE FAILING EXPERIMENTAL HEART

JOHN R. SMITH, M.D., AND JULIUS JENSEN, M.D., ST. LOUIS, MO.

These studies were undertaken to explore, more completely, the effect of theophylline upon the heart in the course of heart failure. Observations were made on dogs.

The heart-lung preparation was used in all experiments. The right and left intra-auricular pressures were measured by direct manometry. The aortic blood pressure was optically recorded by means of a Wiggers manometer. Coronary arterial inflow was recorded using a Gregg-Green differential pressure flowmeter. Cardiac size and output were measured by the usual means. Following control observation, heart failure was induced by the careful addition of chloral hydrate (20 per cent) to the profusing blood until myocardial dilatation and elevation of "venous pressures" were noted. Heart failure was then manifested by cardiac dilatation, fall in cardiac output (from 25 to 50 per cent), elevation of intra-auricular tension (especially that of the left auricle), and gradual decline of blood pressure. Net coronary arterial inflow in the failing heart-lung preparation was usually not altered. Theophylline aminoisobutanol (0.06 Gm.) was then added to the profusing blood.

The administration of the theophylline to the failing heart demonstrated that:

1. The drug has an immediate effect upon the myocardium. Vigor of contraction was greatly enhanced, with concomitant diminution of intra-auricular tensions and decrease in size of the heart.

2. There was immediate diminution of pulmonary congestion.

3. No immediate increase in coronary arterial inflow occurred.

The decrease in pulmonary edema following administration of theophylline aminoisobutanol to the failing heart-lung was so striking that the phenomenon was investigated separately. Using the de Barenne modification of the heart-lung preparation, blood flow through edematous lungs could be measured, eliminating cardiac pulsatile pressure. It was found that:

1. An immediate and striking decrease in pulmonary edema and emphysema occurred after administration of the drug, with free flow of blood through the lungs.

2. Only the pulmonary edema of heart failure responded thus to theophylline aminoisobutanol. Pulmonary edema produced by the inhalation of ethyl acetate or by simple, mechanical chronic passive congestion (without heart failure) did not respond to theophylline.

DISCUSSION

QUESTION.—Has there been any success in the treatment of emphysema without heart failure?

QUESTION.—I would like to ask if any method other than chloral hydrate produced heart failure.

DR. EMMET B. BAY, Chicago, Ill.—I would like to ask what type of reservoir was used in the last experiment. At what pressure was the blood flowing into the pulmonary artery?

DR. SMITH (closing).—There may be some application of this method to the treatment of emphysema without heart failure. It is well known that the effect of this drug upon the lungs, particularly in cardiac asthma, is quite striking. It has been noted to be effective in

bronchial asthma. I would imagine that, if emphysema were complicated by chronic bronchitis, this drug would have some beneficial effect.

Chloral hydrate was the only drug used to produce heart failure.

In answer to Doctor Bay, the type of reservoir used in the last experiment was at fixed elevation (33 cm. above the right auricle). In ordinary heart-lung preparation, this elevation of the venous reservoir assures one of an adequate blood flow into the heart.

EFFECT OF STIMULI ORIGINATING IN THE UPPER ABDOMEN IN THE CAUSATION OF REFLEX SHORTENING OF THE ESOPHAGUS

FREDERICK DEY, M.D. (BY INVITATION), N. C. GILBERT, M.D.,
R. C. ROSKELLEY, M.D. (BY INVITATION), AND RUTH TRUMP, B.S.
(BY INVITATION), CHICAGO, ILL.

There are clinical reasons for assuming that some cases of hiatus hernia may be caused by reflex shortening of the esophagus consequent upon impulses arising from the upper abdomen. In a previous paper, it was demonstrated that electrical stimulation of the vagus nerve caused sufficient shortening of the esophagus to pull the stomach up to the hiatal orifice. In these experiments the chest was closed, and we were not able to pull the cardiac end of the stomach through the hiatal opening. The present series of experiments were performed on the anesthetized dog with the left chest opened and a lever attached to the esophagus. Movements of the esophagus were recorded upon a revolving drum. Manipulation of the liver, pressure upon the gall bladder, and distention of the gall bladder caused a reflex shortening of the esophagus similar to that obtained by electrical stimulation of the vagus. Reflexes were obtained elsewhere in the gastrointestinal tract. A hiatus hernia occurred with slight pressure upon the abdomen before it was opened, and during the experiment with the abdomen opened it was reproduced again by a shortening of the esophagus due to reflex stimulation or electrical stimulation.

GASTROCOLIC FISTULA—AN EXPERIMENTAL STUDY

R. JOHN F. RENSHAW, M.D. (BY INVITATION), R. M. KISKADDON, M.D.,
(BY INVITATION), AND FREDERIC E. TEMPLETON, M.D., CLEVELAND, OHIO

Patients having gastrocolic or gastroenterocolic fistula present a combination of symptoms consisting of nausea, vomiting (often with fecal material), diarrhea, nutritional deficiency, and cachexia. In some patients the stools are clay colored and contain undigested food, neutral fats, and fatty acid crystals. The patients may also develop anemia, sometimes of the hyperchromic macrocytic type. The usual explanation of the syndrome is that food passes directly from the stomach into the large intestine and short-circuits the small bowel. A few workers disagree with this explanation. For instance, Pfeiffer and Kent, on the basis of fluoroscopic observation, record that the presence of undigested food "results from exceedingly rapid transport of food through the stomach and intestine and is probably due to irritation of the tract from the presence of colonic contents from the stomach and bowel." Atwatter, Butt, and Priestly

admit that reflux of fecal content plays a part in causing the syndrome, but they state that the mechanical factor (shunting of food out of the stomach into the colon) is foremost.

In view of these conflicting opinions, we observed the flow of barium mixtures in nine patients and in six dogs having gastrocolic, duodenocolic, and gastroenterocolic fistulas. In four patients, as well as in the experimental animals, the major portion of the aliment passed down the small intestine instead of entering the colon through the fistula. The human small intestine appeared abnormal, as in the so-called "deficiency state." Roentgenologic findings followed the descriptions of most roentgenologists: The gastrocolic fistula may be overlooked by giving a barium mixture orally but is usually recognized during administration of a barium enema. In one dog, at roentgenologic examination, we not only watched the barium leave the stomach through the pylorus and flow through the small intestine and colon, but also observed a portion of the mixture return to the stomach through the fistula and emerge again into the small intestine through the pylorus.

The studies confirm the opinion of Pfeiffer and Kent that in gastrocolic, gastroenterocolic, and duodenocolic fistulas, much of the barium mixture follows the small intestine and does not flow directly into the colon. The deficiency pattern of the small intestine seen by roentgenologic examination, along with the presence of neutral fats and fatty acid crystals which were observed in the stools of three patients and all the dogs, indicates that the small intestine is injured. It is suggested that the fecal current, flowing into the stomach and upper small intestine through the fistula, injures the mucosa so that the intestine cannot perform the normal processes of absorption and secretion. Experiments now in progress are designed to study further the latter possibility.

DISCUSSION

DR. E. L. DEGOWIN, Iowa City, Iowa.—In patients with gastrocolic fistula, when the fistula is repaired, the patient is improved and the condition seems reversible. I wonder if any observations were made and whether any histologic evidence is present to show that the damage produced in the experiments is reversible.

QUESTION.—How long did the dogs survive following surgery?

DR. GEORGE E. WAKERLIN, Chicago, Ill.—Since the dogs used in this study showed a macrocytic hyperchromic anemia, the possibility that this type of animal might serve as a means of assaying preparations containing the antipernicious anemia principle comes to mind. Have you studied the effect of the antipernicious anemia principle in these dogs?

DR. KISKADDON (closing).—In regard to the first question, "Is the pathologic process reversible?" we feel that there is definite evidence, as presented in the slides of the pathologic specimens, that these are chronic ulcers. We do not have evidence, as yet to indicate whether the dogs can be brought back to normal, because we have been primarily interested in the direction of barium flow and the changes which take place in the intestine. We are investigating these dogs gastroscopically and are studying other dogs into which we have introduced permanent cannulas. We thereby hope to be able to observe the formation and retrogression of the ulcerative process.

In answer to the second question concerning the length of life of these dogs with internal fistulous communications, we must explain that it varies with the type of fistulous connection. Dogs with gastrocolic fistulas survive about four months. However, dogs with

gastrojejunocolic fistulas usually survive about two months. The dog with a duodenocolic fistula died one month after the fistula was established. This brings up the point as to what the protective factor may be in dogs with gastrocolic fistulas in contrast to dogs with gastroenteric fistulas. It is possible that in the dogs with gastrocolic fistulas the colonic microorganisms are killed by the acid of the stomach, but we do not know that this is true. In contrast, the acid component would not be present in dogs with gastroduodenal fistulas, and therefore there should be no means of inhibiting the destructive action of the bacterial flora on the upper intestinal mucosa.

As you can see, our experiment is still in its initial phase, and we are unable to answer the last question concerning the availability of such dogs in the bio-assay of the antipernicious anemia factor.

SULFATHALIDINE—FURTHER STUDIES ON CLINICAL, CHEMICAL, AND BACTERIOLOGIC EVALUATION IN INFECTIOUS DISEASES OF THE COLON*

MICHAEL H. STREICHER, M.D., CATHERINE GRENCI (BY INVITATION), AND NANCY CLAAR (BY INVITATION), CHICAGO, ILL.

Experimental evidence obtained shows that sulfathalidine has bacteriostatic properties, that it is nontoxic in man, and that it is superior to any sulfonamide used in the colon. It is effective in doses of 1 Gm. orally three times daily. This dose may be maintained for several months without producing deleterious effects. Prolonged administration and increased doses of this agent do not elevate the blood concentration above from 1 to 1.5 mg. per 100 c.c. of blood.

The chemical determinations of sulfathalidine in the stool (total amounts) tend to show that, when oral intake is increased to 12 Gm. daily, the recovery of the drug in the stool is not increased correspondingly.

Inasmuch as the blood and the urine concentration levels remain unchanged, this experiment tends to show that large doses of sulfathalidine are not utilized in the colon.

Another experiment designed to study the total recovery of the drug in the stool after the intake of the last dose shows that 90 per cent of sulfathalidine is accounted for on the seventh day.

Bacterial counts demonstrated a decrease in total number and in the number of the coliform group, the staphylococci, and the streptococci.

Clinically the patients who are ill with chronic ulcerative colitis, bacillary dysentery, or *Giardia lamblia* may be treated with sulfathalidine to advantage. In amebic dysentery the drug is used to curtail the activity of secondary invaders.

Patients with diarrhea register definite improvement, the stools become more formed and odorless, the number of evacuations are decreased, the blood in the stool disappears, the cramping in the abdomen is alleviated, the tenesmus in the rectum subsides, and in general the patient eats better and feels better.

*The work on this research problem was made possible through a grant sponsored by Sharp & Dohme, Inc., Glenolden, Pa.

DISCUSSION

DR. RALPH C. BROWN, Chicago, Ill.—I think it would be a mistake for the impression to become widespread in the profession that the sulfonamides are of great value in the treatment of nonspecific ulcerative colitis. There is no question about the ability of these drugs to lower the bacterial counts decisively. This was first proved very thoroughly in the case of sulfaguanidine, very careful bacterial counts having been made on a series of cases. For many years at the Presbyterian Hospital we have made an intensive study of a large series of patients with ulcerative colitis. With each successive sulfonamide as it has appeared, we have hoped that finally a therapeutic agent had been devised that would demonstratively affect the course of the disease. However, in our experience we have not been able to convince ourselves that any of the sulfa preparations actually do so. I think that anyone who has had very much experience with nonspecific ulcerative colitis is likely to feel that success in the treatment of these cases depends very largely upon the stage at which they are seen and effectively treated, and when I say effectively treated I do not mean that we have anything even approaching a specific type of treatment. These patients must be treated in much the same manner as those with pulmonary tuberculosis in the sense that they require a high calorie diet and particularly a long period of bed rest, with large amounts of vitamins B and C. If they are seen early in the course of the disease, it will be found that surprisingly good results can be obtained in most cases if the duration of treatment is determined not alone by the clinical condition of the patient, but chiefly by the presence or absence of inflammatory changes in the bowel wall as shown by the proctoscope and by x-ray examination.

DR. STREICHER (closing).—I appreciate the comments of Doctor Brown. I am sure that sulfathalidine alone is not the answer to the problem. We have been working on this problem for many years and have had a good many experiences. In the extremely acute cases we have used blood transfusions, liver extract, and all other supportive measures.

As far as judging the progress of the disease by clinical observation alone, we do not depend on that entirely; we re-proctoscope our patients. Some few years back we published a report in the *Journal of Digestive Diseases* on 200 patients proctoscoped yearly over an eight-year period. I quite agree with Doctor Brown that sulfonamides alone are not the entire answer, but sulfathalidine is the best we have of the sulfa group at this time.

THE PRESIDENT'S ADDRESS BY WILLIS FOWLER was presented at this point in the program.

THE DETECTION OF DANGEROUS CARRIERS OF HEMOLYTIC STREPTOCOCCI BY THE MEASUREMENT OF THEIR "STREPTOCOCCAL OUTPUT"

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Students of streptococcal infection have recognized, for several years, that all throat carriers of beta hemolytic streptococci are not equally capable of transmitting infection to others. However, no means of differentiating "dangerous" from "innocuous" carriers have been available.

A solution to this problem was sought by measuring the number of hemolytic streptococci expelled into the environment by soldiers hospitalized at the Station Hospital, Camp Carson, Colorado, with streptococcal tonsillitis, pharyngitis, and scarlet fever. Contamination of the bed clothing was employed as an index

*These investigations were carried out by the Commission on Air-Borne Infections, Army Epidemiological Board, Preventive Medicine Service, Office of the Surgeon General, United States Army.

of the "streptococcal output" and was measured quantitatively by pinning a small cloth patch to the bottom sheet overnight. The patch was then removed and immersed and shaken in broth, an aliquot of which was used for making a blood agar pour plate. The number of colonies of hemolytic streptococci which developed after incubation represented the "streptococcal output."

This test, performed many times in more than 400 patients, showed that the number of hemolytic streptococci expelled bore no relation to the number of these bacteria on the throat culture plate. However, patients with strongly positive nose cultures dispersed large numbers of the organisms, whereas those with negative nose cultures expelled very few. Hospital cross-infections, infections in barracks, and a food-borne epidemic of streptococcal pharyngitis and tonsillitis were traced to carriers with positive nose cultures who exhibited high "streptococcal outputs."

It is concluded that carriers of beta hemolytic streptococci whose nose cultures are positive represent a more dangerous group than those in whom the streptococci remain confined to the throat.

DISCUSSION

MAJOR F. S. COOMBS, JR., Chanute Field, Rantoul, Ill.—I think that the paper is very stimulating. We, too, have been interested in the same problem. I wonder if there is any difference in the length of the disease when patients are nose carriers rather than throat carriers.

DR. M. A. BLANKENHORN, Cincinnati, Ohio.—I would like to ask whether Doctor Hamburger found carriers in other persons in the hospital. He has studied bed patient carriers, but what about persons who worked about the ward and ministered to these patients?

I would like to ask if he has an opinion regarding the value of the gauze face mask in preventing dissemination.

DR. IRVINE H. PAGE, Cleveland, Ohio.—I would like to ask Doctor Hamburger whether he knows anything about the use of D.D.T. or aerosols of propylene glycol in preventing the spread of hemolytic streptococci. Is there anything known about which Lancefield grouping the streptococci belong to?

DR. HAROLD LUETH, Evanston, Ill.—Was any attempt made to study the susceptibility of persons affected to see whether or not they were uniformly susceptible or whether there was any variation among the group?

DR. HAMBURGER (closing).—In reference to Major Coombs's question as to whether a streptococcal infection has a longer clinical course in a patient whose nose, as well as throat, is positive, we have no specific data on this point. However, in general, hospitalized patients with positive nose cultures tend to be sicker than those in whom the nose is not invaded. At Camp Carson about 65 per cent of the men hospitalized with hemolytic streptococcal infection had positive nose cultures, and some of them expelled great numbers of streptococci during the early stage of their disease.

As to the applicability of these facts to ambulatory, as well as to hospitalized, carriers, they apply to both. Actually, most dangerous carriers are infected individuals. Captain H. M. Lemon and I saw a good many soldiers this spring at Fort Lewis, Wash., who may or may not have had fever but who had mild streptococcic infections and were picked up as nasal carriers on surveys. They had made no complaint, but physical examination and antibody studies showed them to be people who were infected but who were going about as any normal person. The average dangerous nasal carrier disperses large numbers of streptococci for a very short time. On the other hand, there are a few, probably not more than 1 per cent, who continue to expel large numbers of streptococci for weeks or months. We have seen a few

such carriers. Apparently, when epidemics occur, nasal carriers increase in number, and though most are dangerous for a short period only, there are more and more of them.

To answer Doctor Blankenhorn, I think I have covered the point about bed patients. The most detailed studies were necessarily done on hospitalized patients, but we have also been able to follow the carrier state by studying carriers in barracks. The carrier of streptococcus type 46 was picked up on a survey and was entirely asymptomatic.

About masks, the problem of how these streptococci get around is a study all its own. For reasons I have not time to discuss, I do not believe that adequate masking is the answer to the problem. However, there are masks which will effectively prevent the spread of microorganisms expelled during talking, coughing, and, to some extent, during sneezing.

As to propylene glycol, we have been interested in this compound which was originally developed by Dr. O. H. Robertson, the Director of the Commission on Air-Borne Infections. For certain technical reasons, propylene glycol is not the answer. We have studied triethylene glycol in reference to its effect upon hemolytic streptococci in hospital wards, and we have found that it produces a substantial reduction in number of streptococci. To what extent reducing the number of organisms in the air will reduce the incidence of infection requires further study.

The final question regarding the susceptibility to streptococcal infection of the patients who developed cross-infections on the wards is an interesting one. The only real answer we have is statistical. We were dealing with young soldiers all of the same age group, living under the same conditions, and receiving essentially the same training. It is true that under certain circumstances certain types of patients are more susceptible to streptococcal infection; measles convalescents, for example. Some of our cross-infections did take place on measles and German measles wards.

ANTIPNEUMOCOCCUS HYALURONIDASE ACTIVITY IN HUMAN SERUM; RISE IN TITER FOLLOWING PNEUMOCOCCUS BACTEREMIA WITH PURULENT INFECTION OF PLEURA AND SYNOVIA

PRELIMINARY REPORT

ROBERT T. THOMPSON, M.D. (BY INVITATION), AND
M. A. BLANKENHORN, M.D., CINCINNATI, OHIO

The identity of hyaluronidase and spreading factor, in that all hyaluronidases possess the power of spreading, has been described. Since spreading factors have been shown to be important in infection, the in vitro effect of human serum in health and during pneumococcic infection upon the activity of three pneumococcus hyaluronidases was investigated. A modification of the mucoprotein clot prevention test described by McClean in 1943 was used to study the antipneumococcus hyaluronidase activity of human serum.

The serum of fifty healthy human beings was compared with the serum of a patient, A. T., who was convalescing from type II pneumococcus endocarditis. The antitype II pneumococcus hyaluronidase activity of the serum of two healthy persons was equal that of A. T., four were one-half that of A. T., ten were one-fourth that of A. T., sixteen were one-eighth that of A. T., seven were one-sixteenth that of A. T., nine were one-thirty-second that of A. T., and two were one-sixty-fourth of A. T.

From the Department of Medicine and the Department of Biochemistry, College of Medicine, University of Cincinnati.

Serial sera of five patients with pneumonia each showed critical rises in titer of antienzyme activity against three types of pneumococcus hyaluronidase which were synchronous and commensurate and not related to the type of pneumococcus causing the pneumonia. Three enzymes (types I, II and VII) were tested for each patient, so there was a total of fifteen separate rises in titer. Thirteen of these rises in titer were eightfold or greater; two were fourfold. Each of these patients had pneumococcus bacteremia, three with empyema and one with arthritis.

This rise of antipneumococcus hyaluronidase titer could not be provoked by administration of therapeutic antipneumococcic serum in two patients. Negligible amounts of antitype II pneumococcus hyaluronidase activity were found in therapeutic type II antipneumococcic serum.

Bacillus welchii antitoxin which had high anti-*B. welchii* hyaluronidase activity was found to have negligible effect against type I and type II pneumococcus hyaluronidase.

It is concluded that human serum possesses antipneumococcus hyaluronidase activity and that this activity is not type specific. Five patients showed critical rises of antipneumococcus hyaluronidase activity subsequent to pneumococcus bacteremia; three of these had purulent infection of pleura and one of synovia. Therapeutic type II antipneumococcic serum possesses negligible amounts of antipneumococcus hyaluronidase activity. This finding probably applies to other types of therapeutic antipneumococcic serum since the antipneumococcus hyaluronidase activity of human serum was found not to be type specific.

THE EFFECT OF SUPERIMPOSED BACTERIAL PNEUMONIA ON THE SEVERITY OF SUBLETHAL INFECTION WITH INFLUENZA VIRUS*

CARL G. HARFORD, M.D., MARY RUTH SMITH, M.S. (BY INVITATION), AND W. BARRY WOOD, JR., M.D., ST. LOUIS, MO.

It is thought that most of the deaths in the pandemic of influenza of 1918-1919 were due to bacterial pneumonia superimposed on a primary viral infection of the lung and that chemotherapy will be effective in controlling the pneumonia if another pandemic occurs. However, Taylor has shown that reinstillation of fluids into the respiratory passages of mice suffering from a sublethal infection with influenza virus converts the infection into one from which the animals die. Because of this observation he has suggested that the bacterial pneumonia may greatly increase the severity of the viral infection. If such synergistic action occurs, it may be anticipated that treatment in human epidemics will be difficult, since the viral infection is not susceptible to any known chemotherapeutic agent.

In this study, infection with influenza virus was produced in mice by intrabronchial inoculation with standardized dilutions of virus. Several days later, when viral lesions had developed, the mice were allowed to inhale fine droplets

*This investigation was aided in part by the Commission on Influenza, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, United States Army.

of heavy suspensions of virulent type I pneumococci or hemolytic streptococci. The bacterial exposure caused fatal pneumonia and bacteremia in about five days. Normal control animals inhaling the same bacteria survived without clinical disease, but their lungs were shown to have many organisms which disappeared within a few hours. One-half the animals with combined infection were given sulfonamide therapy beginning twenty-four hours after inhalation of the bacteria, that is, after the superimposed bacterial pneumonia was well established. The treated animals survived and showed sublethal viral lesions when examined two weeks later.

In these experiments, superimposed bacterial pneumonia did not convert a sublethal viral infection into a lethal one. Under the conditions studied, the bacterial infection did not have the same effect as the reinstillation of fluid described by Taylor. Furthermore, the bacterial pneumonia in the present of viral lesions was controlled by sulfonamide chemotherapy. These observations suggest that chemotherapy will be of value in treating secondary bacterial pneumonia complicating epidemic and pandemic influenza.

DISCUSSION

DR. H. NECHELES, Chicago, Ill.—We can confirm these results by our experience on dogs with distemper which we have treated for the last ten years with sulfonamide drugs. Distemper in dogs is a virus disease. In the beginning it is a pure virus, but, as soon as the nasal and conjunctival flow begin, we have a mixed infection with pneumococci and cocci of all sorts. These dogs are benefited by sulfonamide, glucose, and saline given subcutaneously. We have been quite successful with this treatment. We believe that, as in the case of the human patient, it is the suppression of the secondary infection which saves the lives of these dogs.

THE SIGNIFICANCE OF THE ACCESSORY SPLEEN

GEORGE M. CURTIS, M.D., AND DAVID MOVITZ, M.D. (BY INVITATION)
COLUMBUS, OHIO

When *accessory spleens* are present in patients with congenital hemolytic icterus or primary thrombocytopenic purpura, the end result of splenectomy is influenced by whether or not they are found and removed at the original operation. This was surmised in 1928 by Morrison, Lederer, and Fradkin for primary thrombocytopenic purpura. Subsequent findings of accessory spleens associated with recurrence further support their original suggestion.

The reported frequency of accessory spleens at necropsy ranges from 10 to 35 per cent and at operations on patients with splenic disease, from 20 to 44 per cent. A greater frequency among the young has been noted.

Of 178 consecutive patients in our series, operated for various splenic disease, fifty-six, or 31.4 per cent, presented accessory spleens. The greatest incidence was found in patients of the first decade with congenital hemolytic icterus, 57.1 per cent. Forty-four per cent were found in patients of the first decade with primary thrombocytopenic purpura. The incidence decreased with age. The number of accessory spleens per patient varied from one to ten, twenty-six patients presenting only one accessory. Single as well as multiple accessories occurred predominantly in the young.

The 131 accessories occurring in fifty-six patients were distributed in the following percentages: hilus, 54.2; pedicle, 25.1; omentum, 12.2; retroperitoneum, 6.1; splenocolic ligament, 1.5; and bowel mesentery, 0.75. Accordingly, these regions are routinely searched subsequent to the actual splenectomy. In the female the left adnexa should be palpated.

Numerous accessories, up to 400, found scattered throughout the peritoneum, including parietal and ventral mesogastrial layers, apparently arise by autotransplantation of splenic tissue scattered about by hemorrhage from a ruptured spleen. Buchbinder and Lipkoff (1939) designated this phenomenon as *splenosis,* distinguishing it from the usual accessory spleens because of the mechanism of formation and the characteristic microscopic structure. As a consequence, splenectomy should be accomplished without spilling splenic tissue, as might occur by tearing the spleen near its adhesions or severing its pedicle too close to the hilus.

Accessories occurred in but a single location in 85.7 per cent, and never in more than two locations. The hilus always constituted one location in each instance of double incidence.

The occurrence as well as the various locations of accessory spleens are clarified by study of the splenic embryologic mechanisms: failure of splenic anlagen to fuse, splenic notches and lobules representing partial fusion, co-existing accessories supplied by the splenic artery, the dorsal mesogastrium subjacent to the splenic area developmentally carrying accessories to distant locations, and the attachment of splenic anlagen to the contiguous genital ridge resulting in scrotal or pelvic accessories.

Two presented cases of recurrence after splenectomy and two more from the literature indicate that unremoved accessory spleens caused the recurrences. Three cases were of recurrent thrombocytopenic purpura, with one accessory observed but not removed and two with pathologically active accessories found at necropsy. One was a recurrent congenital hemolytic icterus relieved by surgical removal of accessory spleens.

A pedunculated accessory spleen may cause acute abdominal symptoms by infarction resulting from torsion of its pedicle. Surgical removal is then indicated.

DISCUSSION

DR. ARMAND QUICK, Milwaukee, Wis.—I should like to ask how often idiopathic thrombocytopenic purpura is seen in children.

DR. OVID O. MEYER, Madison, Wis.—I would like to ask whether or not these accessory spleens are of any importance, other than in the matter of torsion which was mentioned, unless there is hypertrophy? It is our impression that these accessory spleens are often very small and easily overlooked and are not important until months or years later when they hypertrophy. They then have an undesirable function.

DR. MOVITZ (closing).—The frequency of recognition of idiopathic thrombocytopenic purpura partly depends on the practices of certain medical centers. We see it relatively frequently. The primary thrombocytopenic purpuras are among the most common indications for splenectomy.

Regarding the small size of most accessory spleens, it appears to be true that recurrence due to an accessory spleen does not occur except after an interval of time. That is the usual history in those few recurrences in which an accessory spleen was demonstrated. The cases reported have all demonstrated considerable pathologic changes in the accessory. As to the size of the accessory spleen causing the recurrence, some were as small as 2 or 3 cm., and one weighed but 12 Gm. It would appear that the minuteness of size of an accessory spleen does not eliminate it as a cause of recurrence at a future time.

OBSERVATIONS ON THE EFFECT OF MASSIVE DOSES OF IRON GIVEN INTRAVENOUSLY TO PATIENTS WITH HYPOCHROMIC ANEMIA

ANNE TOMPKINS GOETSCH, M.D. (BY INVITATION), CARL V. MOORE, M.D., AND VIRGINIA MINNICH, M.S. (BY INVITATION), ST. LOUIS, MO.

Massive doses of iron (from 0.608 to 1.32 Gm. iron as colloidal ferric hydroxide or colloidal ferric oxide) have been given intravenously in one infusion to eight different patients with hypochromic microcytic anemia. One patient was given a second injection after an interval of four months, so that the administration was made nine times.

The following observations were made:

1. Reticulocyte responses in each instance exceeded those expected with oral therapy. Three additional patients who received smaller doses showed reticulocyte rises higher than the average responses reported by Heath after optimal oral administration. This suggests that "optimal" oral therapy does not provide a maximal stimulus to the outpouring of reticulocytes from the bone marrow. Three control subjects failed to develop a reticulocytosis when given comparable amounts of iron intravenously.

2. The average rate of hemoglobin regeneration per 100 c.c. of blood per day was 0.224 Gm.; the lowest, 0.16 Gm.; and the highest, 0.27 Gm. These figures indicate the rise which occurred from the day of iron administration to the time at which the rate of hemoglobin increase was obviously becoming slower. Since correction was not made for blood lost by three of the patients during the recovery period, figures for the rate of hemoglobin formation are lower than they otherwise would have been. They are distinctly greater than those usually observed following oral therapy but no greater than is found in an occasional patient. The data suggest that the fastest rate of hemoglobin regeneration which can be stimulated by iron in patients with hypochromic anemia approximates 0.3 Gm. per 100 c.c. per day.

3. Calculations indicated that from 71.8 to 99.7 per cent of the injected iron was used for the synthesis of hemoglobin. These figures are lower than they would have been if several of the patients had not lost blood during the recovery period. The observation of other workers that parenterally administered iron is almost completely retained by the body and converted into hemoglobin was confirmed.

4. Toxic reactions to the injected iron were mild or absent in two patients. In most instances the patients developed nasal stuffiness, paresthesias, palpitation, nausea, transient fall in blood pressure, and, later, manifestations of fever and weakness. In three instances these symptoms progressed to an alarming degree, and therapy was interrupted. In all patients the toxic symptoms subsided within a few hours. The toxic manifestations to massive intravenous iron administration are great enough to contraindicate its therapeutic use.

DISCUSSION

DR. IRVINE PAGE, Cleveland, Ohio.—What would be the effect if you used an organic iron preparation, ferric gluconate, and not a colloid? Would part of the effect be due to the introduction of the colloid particles as well as to the iron in solution?

QUESTION.—What is the pH of the injected fluid?

QUESTION.—I should like to ask whether there were variations in the rate of administration and what the rate was.

QUESTION.—I would like to ask if you have seen patients who were so intolerant of oral administration that they would consider the parenteral use of iron in smaller doses.

DR. H. NECHELES, Chicago, Ill.—Have you information about a French parenteral iron preparation? It was a water-clear solution and, as I remember, the dose was quite small. Colloidal iron is quite a strong protein precipitant. Is the reticulo-endothelial system affected?

DR. GOETSCH (closing).—"Clear iron," or metallic iron, in simple solution has been used in small doses for parenteral injection, usually by the intramuscular route, although it has also been given intravenously. Toxic effects observed following only from 5 to 10 mg. doses intramuscularly were similar to those we have observed using colloidal iron; this is one basis for our impression that these toxic effects are so-called "heavy metal effect" rather than a result of the colloidal suspension per se. When simple iron solutions are given to dogs in doses of 1 mg. or more per kilogram of body weight, twelve hours or more are required for serum iron values to return to normal, whereas the return to normal is prompt after colloidal iron has been given. This more rapid disappearance of colloidal iron from the blood may explain the fact that massive doses could be given to our patients in a single intravenous injection.

Animal experimentation has shown that the reticulo-endothelial system is the site of storage of injected iron. We did no studies to demonstrate blocking of the reticulo-endothelial system in our patients. There were no demonstrable emboli. One patient complained of severe chest pain during the administration of iron, but no signs of pulmonary embolism appeared.

The solutions contained approximately 3.5 mg. per cubic centimeter; their pH varied from 3.5 to 3.8; the rate of administration was from 10 to 25 drops per minute; and from three to five hours were required for administration of total dose.

Reticulocyte responses have been reported after very small doses of iron, 10 mg. in several instances. We found this to be true in our three patients who had toxic reactions necessitating discontinuation of the injection. In these cases it was possible to obtain a second reticulocyte peak with subsequent oral therapy. Significant rise in hemoglobin was not observed after small doses, since any apparent rise was within the usual fluctuations in hemoglobin levels. Maximum reticulocytosis was observed only following the larger doses of iron.

The toxic effects of small doses of colloidal iron, either intramuscularly or intravenously, are not great enough to contraindicate its use but are unpleasant enough to suggest that its use be reserved for the rare patient who cannot tolerate oral iron therapy. Furthermore, it was our experience that small intravenous injections caused thrombosis of the vein as readily as did larger doses.

THE ACTION OF VARIOUS DRUGS ON THE PROTHROMBIN OF THE BLOOD

ARMAND J. QUICK, M.D., MILWAUKEE, WIS.

In order to test whether large doses of synthetic vitamin K will increase the prothrombin of the blood, menadione and two water-soluble derivatives, Hykinone* and Synkayvite† were administered to rabbits, dogs, and human beings, both orally and intravenously. No change in the prothrombin was observed after giving one large single dose.

In a subject who has a consistent prothrombin a little less than 50 per cent of normal (from fifteen and one-half to sixteen seconds) but is otherwise entirely normal and never has had abnormal bleeding, 40 mg. of hykinone intravenously had no effect on the prothrombin level. The mother and a sister likewise have a prothrombin of 45 per cent. This indicates that the condition of a fixed lowered level of prothrombin can be hereditary and congenital. The prothrombin concentration of the blood, whether normal, congenitally low, or reduced by permanent liver damage, is fixed and apparently cannot be altered even by large doses of synthetic vitamin K.

Link and his associates have reported that caffeine, theobromine, and theophylline bring about a hyperprothrombinemia and also counteract the hyperprothrombinemic action of dicumarol. In the present study it was found that single doses of 200 mg. of the three methylxanthines neither elevated the prothrombin nor inhibited the action of dicumarol in rabbits and dogs.

Recently, Pirk and Engelberg published results showing that daily doses of 0.33 Gm. of quinine sufate given to normal individuals caused a drop in the prothrombin time of from five to twelve seconds. These authors used Russell viper venom as the thromboplastic reagent for determining prothrombin. In the present work in which my method for prothrombin was used, no reduction in prothrombin of the blood was found after administering 0.33 Gm. of quinine sulfate daily for eight days.

The discrepancies in results are probably due mainly to differences in the procedures employed for determining prothrombin. It is questionable whether true hyperprothrombinemia is ever found or can be induced. All present evidence so far advanced for it is based on the prothrombin time of highly diluted plasma, which is necessarily artificial and empirical since the physicochemical equilibrium is profoundly disturbed.

DISCUSSION

DR. FREDERICK DEY, Chicago, Ill.—We were quite concerned about the recent report that methylated xanthines are capable of increasing the coagulability of the blood in the experimental animal. We use these drugs quite extensively in the treatment of cardiac diseases, and while we have had no reason for feeling that they promoted thrombosis in our patients, we thought it only wholesome to study the effect of these drugs upon the clotting mechanism of the human being.

*Abbott Laboratories, North Chicago, Ill.
†Hoffmann-La Roche, Inc., Nutley, N. J.

A group of normal patients who had never before received methylated xanthines were admitted to the hospital. During the initial week of the study, daily clotting times, heparin curves, and prothrombin times, using both whole and diluted plasma, were run on the patients. After the normal was established, methylated xanthines were administered by various routes for a period of one week. Again, daily studies of the clotting times, heparin curves, and prothrombin times were made. We found no variations from the normal after the administration of these drugs.

Similar studies of clotting activity were made on a group of fifteen patients who had been taking methylated xanthines for a period of six weeks up to twelve years. Again, there was no variation from the normal.

Finally, we repeated the work on dogs, similar to the study of Doctor Quick's, and found no change in clotting activity.

DR. GEORGE E. WAKERLIN, Chicago, Ill.—In a study made for another purpose, our research group administered vitamin K in a daily oral dose of 60 mg. for six months to six dogs with renal hypertension. Limited studies of the prothrombin levels of these animals showed no significant change from the normal.

DR. E. V. ALLEN, Rochester, Minn.—This question is outside the proposition, but I would like to ask Dr. Quick what was the effect of the drugs in producing hypoprothrombinemia?

MAJOR FREDERICK S. COOMBS, JR., Chanute Field, Rantoul, Ill.—I should like to answer Doctor Allen's question, if I may. We have given large intravenous and oral doses of salicylate and have followed the prothrombin time in a number of patients with rheumatic fever. We have found that there is a slight, but definite, increase in the prothrombin time which is proportional to the plasma salicylate level. However, the prothrombin time increased from a normal of 13 seconds to as high as 20.5 seconds when the plasma salicylate level approaches 70 mg. per cent. In a second group of well persons the administration of 6 Gm. of salicylate per day for more than a week did not affect the prothrombin time, either when measured on whole or 12½ per cent plasma.

DR. OVID O. MEYER, Madison, Wis.—We have had a running controversy for two or three years on this matter of the use of dilute or whole plasma in the determination of the prothrombin time. Professor Link uses dilute plasma because he argues that he can detect minor, but significant, changes. In the clinic we felt that if we could not detect change with whole plasma, the changes were not clinically significant. We want as great simplification as possible. In this matter of the use of xanthines in the animal, Link has stated that you get hyperprothrombinemia and, by inference, that clotting may be favored and thus an undesirable effect on the coronary circulation result, rather than a beneficial effect. I think that with careful analysis one would be of the opinion that hyperprothrombinemia, whether it exists or not as a result of xanthine administration, is not of such order that one might fear it in the usual clinical practice. Another problem is that of hemorrhage possibly resulting from the administration of salicylates. I make a particular point of this because again we have some disagreement. Clinical reports to date arguing for the important role of salicylates in the production of hemorrhage are not too impressive. Hypoprothrombinemia produced by salicylate administration to man is ordinarily mild and in itself does not seem to be of a degree to result in hemorrhage.

I have one question for Doctor Quick and that is on the matter of congenital hyperprothrombinemia. I wonder whether or not this may be a matter of thromboplastin abnormality? Prothrombin is a pretty vague substance and, unless you use several types of thromboplastin, might it not be possible that the blood of this patient would have an effect on the thromboplastin in such a way that we would have an abnormal result irrespective of the prothrombin level?

There is another question. I noted that in this student weighing 188 pounds you used 40 mg. of vitamin K. I wonder if when you have heavy individuals you might not have to use 60 or 80 mg. for the desired results?

DR. L. N. KATZ, Chicago, Ill.—I would have wished that Doctor Quick had found that the xanthines had accelerated the prothrombin time. Implicit in the discussion is the implication that these drugs are valuable in the treatment of coronary disease. I simply wish to state that the burden of proof is still upon those who maintain this is so as far as oral xanthines are concerned.

DR. QUICK (closing).—In the studies which I have made on the administration of large doses of vitamin K orally in animals, I have noted no particular change in the clotting time or in the concentration of prothrombin. When one gets abnormal results such as clotting before the blood can be decalcified, it is difficult to offer any satisfactory explanation.

I think that Major Coombs has pretty well answered the question concerning salicylates, and the remarks of Doctor Meyer have also answered it. Recently it has been claimed that quinine causes hypoprothrombinemia. I have repeated those experiments and have been unable to find any decrease in prothrombin after quinine. We are probably going to discover in addition to dicumarol and the salicylates other drugs that will reduce prothrombin. The sulfonamides which suppress the bacterial flora of the intestines can in certain individuals reduce the available vitamin K sufficiently to cause a serious hypoprothrombinemia.

The inquiry concerning thromboplastin brings up the question as to what the ideal reagent for activating prothrombin is. I have found dehydrated rabbit brain consistently satisfactory, and I have reason to believe that it is as close to the natural activator as any other known natural material.

I think that the case of hypoprothrombinemia which I have presented is a true congenital one and that the defect has gone through a number of generations. I think that if we would examine large groups of people we would find an occasional case of primary hypoprothrombinemia. I am not qualified to discuss the use of xanthines in heart disease.

THE EFFECT OF THIOURACIL ON LEUCEMIA WITH A CLINICO-PATHOLOGIC REPORT OF A CASE OF CHRONIC MYELOID LEUCEMIA THAT DEVELOPED AN EXTREME NEUTROPENIC LEUCOPENIA

LOUIS R. LIMARZI, M.D., CONRAD L. PIRANI, M.D. (BY INVITATION), AND RICHARD J. KULASAVAGE, M.D. (BY INVITATION), CHICAGO, ILL.

One of the toxic reactions of therapeutic doses of Thiouracil in the treatment of hyperthyroidism is the production of a granulopenia. Both a nonfatal and fatal type of agranulocytosis following administration of thiouracil have been reported. The selective action of thiouracil on the granulopoietic tissue is the basis for the therapeutic evaluation of the drug in leucemia. Four cases of chronic myeloid leucemia and one case of chronic lymphatic leucemia were treated with the drug.

The total amount of thiouracil given varied from 1.6 Gm., administered in divided doses over a period of eight days, to 274 Gm., given in divided doses over a period of three months. The average daily dose ranged from 0.2 to 3.0 Gm. In three cases of myeloid leucemia and in the case of lymphatic leucemia, the drug was without effect on the leucemic process in either the blood or bone marrow, basal metabolic rate, or blood chemistry. There was no clinical improvement. In one of the cases of myeloid leucemia, with specific cutaneous

lesions that terminated with an acute blood pattern (''hiatus leukemicus''), the hemopoietic, as well as the other, organs showed a general infiltration of tissue by myelogenous cells of all types. There was no morphologic thyroid hyperplasia. In another case of myeloid leucemia, in which a remission was established with roentgen treatment and liquor potassii arsenitis (Fowler's solution), 0.8 Gm. of thiouracil daily for a period of three months failed to maintain this remission or prevent a relapse of the leucemic process.

A fourth case of leucemia in a Negro woman, 50 years of age, with a leucocyte count of 440,000 and a chronic myeloid blood pattern, was maintained on a daily dosage of 3.0 Gm. No other therapy for the leucemia was given. In three months the leucocyte count gradually dropped to 60,000, and the drug was discontinued. The blood pattern was still that of a chronic myeloid leucemia. There was no improvement in the clinical condition nor a decrease in the size of the enlarged spleen. The basal metabolic rate was still elevated, and there were no blood chemical changes. In the five weeks that followed, the total white count gradually dropped to 1,900, with a differential count consisting of 18 per cent myeloblasts, 33 per cent basophiles, and 49 per cent lymphocytes. There was a complete absence of neutrophiles and eosinophiles in the blood. The blood uric acid gradually increased to 11.4 mg. per 100 c.c. of blood. The patient developed toxic disturbances of the central nervous system consisting of convulsive seizures, myoclonic contractions of various muscles, particularly of the facial muscles, and frequent and severe stages of somnolence and confusion. At autopsy the bone marrow showed numerous myeloblasts and lymphocytes and a complete aplasia of mature and immature types of neutrophiles and eosinophiles. All the organs, including the hemopoietic tissue, showed a minimal amount of leucemic infiltration consisting of myeloblasts and a conspicuous lymphocytic reaction. The hemopoietic organs revealed a marked increase in reticuloendothelial elements. No histologic evidence of a thyroid hyperplasia was observed in biopsy material taken during the administration of thiouracil or at autopsy. Except for a few myeloblasts and lymphocytes in the blood vessels, the brain tissue was free of any leucemic infiltrations. It is concluded that (1) large daily doses (3.0 Gm.) of thiouracil for a period of three months will produce an extreme neutropenic leucopenia with a minimal leucemic infiltration in the organs and a complete aplasia of neutrophilic elements of the bone marrow; (2) thiouracil produces toxic disturbances of the central nervous system; (3) in cases of myeloid and lymphatic leucemia small doses (0.8 Gm. or less daily) of thiouracil for short or long periods of time is without effect on the leucemic state; (4) in cases of chronic myeloid leucemia with a high percentage of basophiles in the peripheral blood, the basophiles are resistant to the toxic effect of thiouracil and in this respect simulate the blood pattern observed in remissions induced by roentgen therapy; (5) there is no clinical improvement in thiouracil-treated cases of leucemia; (6) thiouracil in sufficient doses regularly inhibits granulopoiesis and destroys granulocytes, an effect which is independent of individual susceptibility.

REACTIONS FROM UNIVERSAL DONOR BLOOD

ROBERT C. HARDIN, M.D., IOWA CITY, IOWA
(INTRODUCED BY WILLIS M. FOWLER, M.D.)

It is the established practice in transfusion to use donor blood of the same A-B type as that of the recipient. Unquestionably, this procedure is theoretically more correct than the administration of universal donor blood without regard for the patient's blood group. It is probably also true that the danger incident to the use of universal blood has been exaggerated.

Recent experience in the Army Medical Service affords the opportunity to make certain comparisons between the reaction rates encountered in transfusion of group-specific blood and universal donor blood. Two series of transfusions can be compared; the first is composed of 7,299 transfusions of group-specific blood and, the second, of 9,392 transfusions of universal donor blood. In the first, a reaction rate of 4.22 per cent was encountered and, in the second, a rate of 4.80 per cent. The universal donor blood was not screened for high agglutinin content.

Because the mechanism by which reactions peculiar to universal donor blood arise is the introduction of an agglutinin into a recipient possessing the corresponding agglutinogen, one is particularly interested in the frequency of hemolytic reactions. The rates in the two series were equal, being 0.20 per cent and 0.18 per cent, respectively.

Mild hemolytic reactions might easily be classified clinically as pyrogenic. Therefore, the difference of 0.50 per cent in pyrogenic reactions between the two series (3.18 per cent and 3.68 per cent) merits closer scrutiny. The rate of pyrogenic reactions was computed for each of the twenty-three weeks during which the study was made. In the first two weeks the use of universal donor blood was characterized by a high reaction rate (9.09 per cent and 7.72 per cent). Subsequently, this class of reactions was equally frequent in both series. The sharp decrease in pyrogenic reactions is evidence that some other mechanism than intravascular hemolysis operated, since reactions on that basis would tend to maintain their frequency. The fall in reaction rate followed the correction of errors in the technique in preparation of diluent fluids.

Conclusion.—No essential difference in reaction rates in two series of transfusions, one of group-specific blood and the other of universal donor blood, was encountered.

DISCUSSION

DR. C. C. STURGIS, Ann Arbor, Mich.—I would like to ask if there were any fatal reactions in these two groups which could be ascribed to the blood transfusion?

QUESTION.—How do you differentiate between pyrogenic and allergic reactions, and what is the time of the reaction following transfusion?

DR. ROBERT W. HEINLE, Cleveland, Ohio.—I certainly agree that universal donor blood can cause trouble at times, but certainly such reactions are uncommon. When it is difficult to get Rh-negative blood, we use a universal donor. When we do use universal donor blood, we set up a minor cross match with the donor's serum diluted 1:100. If no agglutination results

with 1:100 dilution, we feel that it is safe to go ahead with type 0 blood because the titer is not sufficient to cause trouble. If the titer is sufficient to show up in a 1:100 dilution, it is evident that we are dealing with an unusually high titer and that the blood should not be used.

DR. HARDIN (closing).—The death rate following transfusions, but not necessarily due to the transfusion, was .06 per cent. The problem of the differential diagnosis of transfusion reactions is one which always causes considerable trouble. Allergic reactions are usually classified as those characterized by urticaria or asthma. Pyrogenic reactions are differentiated from hemolytic reactions by drawing from the patient from 3 to 5 c.c. of blood, centrifuging, and inspecting for free hemoglobin in the plasma. That type of test will not rule out all kinds of hemolytic reactions, particularly the Rh reaction in which the hemolysis may be delayed. The usefulness of the time factor in transfusion reactions is also open to question because sometimes the phenomena of the hemolytic reaction may appear late. However, in the true A-B incompatibility, the symptoms usually appear before the first 50 to 75 c.c. of blood have been administered.

EFFECT OF RESUSPENDED ERYTHROCYTE TRANSFUSION ON SERUM PROTEIN

SAMUEL G. TAYLOR, III, M.D., AND FRANCIS LYTLE, M.D. (BY INVITATION)
CHICAGO, ILL.

The presence of hypoproteinemia is commonly considered a contraindication for resuspended red cell transfusion as a substitute for whole blood.

Total protein determinations were made on patients immediately before and twenty-four hours after resuspended red cell transfusions. In patients who showed a marked anemia and hypoproteinemia, a definite rise in serum protein level generally occurred in twenty-four hours. In patients with hypoproteinemia, but no anemia, no significant change in serum proteins could be demonstrated despite repeated resuspended red cell transfusions sufficient to produce a polycythemia state. Most patients with anemia and serum protein levels within a normal range showed a very slight rise or no change in serum protein level, while a few patients showed a slight drop.

Dogs made hypoproteinemic by plasmapheresis showed no appreciable rise in serum proteins following large repeated red cell transfusions during the period of experiment.

It appears from these studies that the incomplete protein contained in erythrocytes (mainly hemoglobin) is not available in appreciable amounts for conversion into plasma proteins during the period of our observations.

The rapid rise in plasma protein demonstrated in patients with anemia and hypoproteinemia may be explained on the basis of improvement in the metabolism in the liver by correcting the anemia and the protein sparing action caused by relieving the demand for hemoglobin production.

DISCUSSION

DR. DONALD D. KOZOLL, Chicago, Ill.—We were interested in treating patients with hypoproteinemia, without anemia, by the use of red cell suspensions washed twice with dextrose and then rediluted with dextrose. Although we did see an improvement in the total serum protein, it did not occur until we gave more than two liters of red cells.

We further checked the effect of red cell suspension on the albumin-globulin ratio and found that wherever there was a rise in the total serum protein, it occurred in the globulin fraction. Appreciating the greater importance of an improvement in the albumin fraction than the globulin, we have been skeptical of the efficacy of this method of treating protein deficiencies.

DR. WARREN B. COOKSEY, Detroit, Mich.—With the decline of enthusiasm concerning the use of suspended red cells, it is not, therefore, such an important subject. I would not agree to that thought, however, because it seems to me that the increasing use of blood is going to make it necessary for us to conserve blood supply, and that view may well hurt the increasing use of plasma fractions. Certainly there are programs in the air at this time in which the application of albumin-globulin ratios, etc., is a part of postwar planning for blood supply. We, in Michigan, particularly are interested in what the future blood supplying agencies will have. We are thinking very much in terms of salvaging cells. In Detroit some of us connected with the Red Cross had an unusual opportunity with Parke, Davis & Company of this same city to salvage a good many red cells, and we supplied 26,000 pounds of these suspended cells to different hospitals. We had no opportunity to make detailed studies. On the other hand, we did some protein studies and found that in some cases supplied quickly with red blood cells there was a rise. Our hospitals which received these cells varied a great deal. Some were hospitals caring for patients with chronic tuberculosis, some caring for patients with other chronic diseases, as at Eloise, which has a great many patients with cancer. The opinion was that these cells certainly were a most useful adjunct to treatment. The statement was made that the patients had an increased sense of well-being and that they really improved. People who were tired and not able to get out of bed were improved and able to get out of bed quickly with massive transfusions. Whatever the answer is in regard to what happens to the protein portion of the blood, we still feel that transfusions can serve a most useful purpose. It was our very good opportunity to supply it in large quantities, and we had very enthusiastic support.

THE SYNDROME OF HYPERVENTILATION

GEORGE L. ENGEL, M.D., EUGENE B. FERRIS, M.D., CHARLES D. STEVENS, PH.D. (BY INVITATION), MYRTLE LOGAN, M.D. (BY INVITATION), AND JOSEPH P. WEBB, M.D. (BY INVITATION), CINCINNATI, OHIO

Involuntary hyperventilation is a not uncommon manifestation. It may occur as a hysterical symptom, as a physiologic concomitant of the emotions of fear or anger, or as the result of diffuse or focal encephalopathy. The symptoms commonly experienced during hyperventilation include numbness and tingling of the extremities and face, buzzing in the head, decreased consciousness, and tetany. Rarely actual syncope occurs.

The mechanism of some of the symptoms has been elucidated in normal subjects by study of the E.E.G.*; arterial and internal jugular venous blood gases, sugar, and pH.; and pulse and blood pressure during voluntary hyperventilation. It was found that different individuals differed in the ease with which different symptoms developed, even though the largest part of the change in arterial CO_2 content and pH was attained within a minute, was comparable in all subjects, and changed but slightly thereafter. Reduction in the level of consciousness occurred readily in some individuals and infrequently in other individuals, but in each individual the disturbance in consciousness was greatest (1) with lower blood sugar, (2) with low oxygen tension of the inspired air,

*Electroencephalograph.

and (3) in the upright position; conversely high blood sugar, high oxygen tension, and the recumbent position protected consciousness. In all instances, the reduction in consciousness correlated with the degree of slowing of the E.E.G.; with marked slowing, unconsciousness occurred; with slight slowing, there was no demonstrable reduction in the level of awareness. When consciousness became markedly reduced, the subject automatically stopped overbreathing and did not start again until E.E.G. slow waves had disappeared.

Study of the O_2, CO_2, glucose content, of pH of the internal jugular blood, and of arteriovenous differences failed to reveal any correlation with E.E.G. slowing or changes in consciousness. This is contrary to the findings of Gibbs, Gibbs, Lennox and Nims.* It was discovered that blood taken simultaneously from both internal jugular veins followed two entirely different patterns in more than one-half of the cases. When the changes in both jugular veins were the same, either pattern could be found. Pattern I included: (1) marked drop in O_2 content, (2) slight drop in CO_2 content, (3) slight rise in pH, and (4) marked drop in sugar content. Pattern II included (1) slight fall in O_2 content, (2) marked fall in CO_2 content, (3) moderate rise in pH, and (4) slight fall in sugar content. These differences are thought to result from individual anatomical variations in the venous contributions to the left and right internal jugular veins, respectively, and account for the failure to correlate the electrical activity of the cortex with the chemical changes. Different portions of the intracranial contents apparently behave differently in regard to venous drainage, blood flow, and metabolism, and it is difficult to evaluate the contribution of the cerebral cortex to internal jugular venous blood in any instance.

Tetany and the subjective symptoms of numbness and tingling were found to occur after longer periods of hyperventilation and, hence, were more common and severe under circumstances where consciousness was retained. In general, if slow E.E.G. waves did not appear within three minutes of vigorous hyperventilation, they were unlikely to appear, while tetanic phenomena became more prominent. In prolonged mild hyperventilation, tetany was common, whereas disturbances in consciousness were unusual. In such instances, hyperventilation was usually involuntarily terminated by muscular fatigue and by tetany of the respiratory muscles.

Actual syncope, with unconsciousness and falling, occurred only in very anxious persons in whom the unpleasant symptoms led to typical vasodepressor syncope, in hysterical patients who also had hysterical syncope, and in rare individuals with poor postural adaptation. In normal individuals blood pressure and pulse showed only slight change in the recumbent position and but moderate narrowing of the pulse pressure and tachycardia in the erect posture.

Syncope due to peripheral circulatory collapse is not a normal component of the hyperventilation syndrome.

DISCUSSION

DR. EDWARD H. RYNEARSON, Rochester, Minn.—I was much interested in your comments on hyperventilation. I think many physicians have had patients whose condition was at first

*Arch. Neurol. & Psychiat. 47: 879, 1942.

considered to be hyperinsulinism but later discovered to be hyperventilation. In one such case that I recall, the level of blood sugar was 40 mg. per 100 c.c. I would like to ask what the lowest blood sugar is that you have encountered.

Two statements can be made about this matter: first, that hyperventilation often can be incorrectly diagnosed as hyperinsulinism; second, that I can remember no instance in which true hyperinsulinism was diagnosed as hyperventilation.

DR. C. C. STURGIS, Ann Arbor, Mich.—I observed a patient with hyperinsulinism who developed hyperventilation under excitement, with the classical changes of tetany in the hands.

DR. GEORGE E. WAKERLIN, Chicago, Ill.—I should like to hear Doctor Engel discuss the relation of blood pressure changes in his subjects to the hyperventilation syndrome. Previous studies have indicated that hyperventilation produces a hypocapnia with a resulting decrease in the tonus of the vasomotor center consequent upon a central action and reflex effect of the hypocapnia upon the vasomotor center. The resultant lowering of arterial blood pressure has been previously considered as playing an important role in the production of altered cerebral cortical activity in hyperventilation.

DR. FORD K. HICK, Chicago, Ill.—Can normal subjects continue hyperventilating for very long? I wonder what would be the effect of a number of cumulative and severe episodes of hyperventilation.

DR. ENGEL (closing).—In answer to the question about blood sugar, the blood sugar levels were determined in internal jugular venous blood. When blood from the arm vein is used, one finds very little change in the blood sugar level. The blood sugar level in the arterial blood may rise, or fall slightly, so actually total hypoglycemia is not an important phenomenon, although it would seem that sugar may be used more rapidly by the brain.

In regard to hyperventilation during hypoglycemia, I think that would occur occasionally because a patient experiencing mild disturbances in consciousness, such as one sees in hypoglycemia, becomes anxious, and anxious people are likely to hyperventilate.

In regard to changes in blood pressure in the different postures, we studied blood pressure in all patients, and there was no correlation between changes in blood pressure and disturbances in the level of consciousness. In the recumbent position the blood pressure is likely to change very little, and disturbances in consciousness may be marked in the recumbent position. In the erect position, there may be some fall of blood pressure and some narrowing of pulse pressure but not to a marked degree. Some patients when first experiencing these distressing symptoms may become frightened and develop vasodepressor syncope. In these instances, one sees a period for a minute or two after hyperventilation has stopped during which blood pressure is maintained and then the development of typical vasodepressor syncope with falling blood pressure and loss of consciousness if the patient is erect. During the marked disturbance in consciousness with hyperventilation, the patient usually does not fall. That is one of the reasons why the disturbance in consciousness has not been as apparent to most observers as we have described it.

The length of time the patient can hyperventilate is limited by three things: (1) loss of consciousness, during which the patient usually stops hyperventilating involuntarily; (2) tetany, which inhibits the action of the respiratory muscles; (3) fatigue. We usually allowed from a ten- to twenty-minute rest period between experiments when we carried out more than one experiment on the same subject. I do not think this accounts for the differences we observed, because we varied the order of experiments in most instances.

METABOLIC AND PHYSICAL CHANGES ASSOCIATED WITH THE ORAL AND INTRAVENOUS ADMINISTRATION OF AMINO ACID PREPARATIONS TO MAN

CHARLEY J. SMYTH, M.D., CARL A. MOYER, M.D. (BY INVITATION), AND ANDREW G. LASICHAK, M.D. (BY INVITATION), ELOISE, MICH.

This study was conducted upon eight active male adults. Each subject was offered a standard 3,544 calorie diet. Following control periods on this diet, amino acid solutions* were given as supplementary nitrogenous food either orally and/or intravenously (equivalent to 100 Gm. of protein per day) in 480 c.c. of sterile water three times daily. The rate of infusion varied from 10 to 40 Gm. per hour.

The following determinations were made: urinary volume, pH, total nitrogen, ammonia nitrogen, chlorides and creatinine; blood chlorides, CO_2 combining capacity, and hematocrit; and body weight. The only consistent changes observed during the administration of amino acid preparations I and II were an elevation of total nitrogen and ammonia nitrogen excretions.

The subjective changes noted during the injection periods were dizziness, nausea, vomiting, anorexia, and weakness. The severity of these was directly related to the rate of administration. However, relatively severe symptoms were noted with relatively slow rates of injection (15 Gm. in two hours) of preparation II.

The objective changes during and subsequent to the injection periods were listlessness, apathy, irritability, and dependent edema. There was no apparent correlation between the subjective and objective observations and blood and urine chemical changes.

DISCUSSION

DR. W. S. HOFFMAN, Chicago, Ill.—It would be very unfortunate if one gained the impression that distressing symptoms are likely to follow the injection of any of the available commercial preparations of amino acids. At the Cook County Hospital we have acquired a great deal of experience during the last two years with parenterally injected amino acid, particularly with Parenamine. We have made injections into hundreds of patients. In many cases we have given as much as 135 Gm. of amino acids a day for as long as three weeks. Very seldom have our patients experienced any untoward symptoms with the injection, and then only when the injections were made at rates higher than 300 c.c. of a 45 per cent solution per hour, or when the patient had a hepatitis. We have data to be published which indicate that if the plasma amino acid nitrogen levels rise well above 10 mg. per 100 c.c., nausea and vomiting may appear, but usually not otherwise.

In the early enthusiasm for the use of parenterally injected amino acids, the administrations were made too rapidly and in too great a quantity in an attempt to build up patients with protein deficiency to normal in as fast a time as possible. We know now that in patients with severe protein deficiency in which as high as from 450 to 500 Gm. of nitrogen had been lost, it is futile to expect a restoration to normal by parenterally injected amino acids alone. The best that can be accomplished is a slight positive nitrogen balance until these injections can be supplemented with orally administered protein. Under such circumstances, 60, 90, or, at the most, 120 Gm. of amino acids should be given per day, at a rate of 15 Gm. per hour. There should be no difficulty, then, especially if hypertonic solution can be avoided.

*Preparation I "Amigen," Mead Johnson and Company, Evansville, Ind.
Preparation II "Parenamine," Frederick Stearns & Company, Detroit, Mich.

We have made a study of blood chemical changes during and after intravenous injections of amino acids which we are about to publish, and we can confirm Doctor Smyth's findings that the changes are insignificant, except for the rise in plasma amino acid nitrogen. One of the unusual findings was a drop in inorganic phosphate comparable to what one sees after glucose injection.

DR. G. E. WAKERLIN, Chicago, Ill.—As I understand it, Amigen is an enzymic casein digest containing 70 per cent amino acids and 30 per cent peptides, whereas Parenamine is an acid hydrolysate consisting entirely of amino acids. Does Doctor Smyth have any explanation for the depression of appetite produced by Parenamine in contrast to Amigen? In view of Doctor Hoffman's comment, the higher amino acid content of Parenamine may be a factor.

DR. SMYTH (closing).—I think it is well to point out that the rates we have used have been two or three times as great as advocated by commercial houses. Our interest in the problem arose when it was observed that patients did not eat the meal following the administration of amino acid mixtures.

With reference to Doctor Wakerlin's question, I have no answer as to why one mixture depresses the appetite more than the other. We are at present investigating a mixture of the ten essential amino acids commercially prepared.* The effect of fortifying this mixture with aspartic and glutamic acid is being studied. Madden and Whipple have shown that these two amino acids are the cause of gastrointestinal symptoms in dogs.

PHOSPHOLIPIDS OF LIVER AND BLOOD STUDIED WITH RADIOACTIVE PHOSPHORUS

JESSE L. BOLLMAN, M.D., AND EUNICE V. FLOCK, PH.D. (BY INVITATION)
ROCHESTER, MINN.

After a single administration of radioactive phosphorus as disodium phosphate to rats, this substance rapidly enters the liver and furnishes a label for the inorganic phosphate of the liver. The radioactivity of the phospholipid of the liver gradually approaches that of the inorganic phosphate of the liver. Calculations based on the exchange of radioactive phosphate from the inorganic fraction to the phospholipid, from analysis made at intervals from thirty minutes to eighteen hours, indicate that approximately 3.5 mg. of P forms new phospholipid in 100 Gm. of liver each hour. The total amount of phospholipid P, from 120 to 140 mg., remains unchanged, so that an equal amount leaves the liver or is metabolized. Approximately 0.8 mg. enters 100 c.c. plasma each hour as phospholipid, and an equal amount leaves the plasma, the normal value of from 3.5 to 4.5 mg. remaining unchanged. These figures account for enough phospholipid turnover in the liver and plasma to metabolize or transfer fat equivalent to only 3 per cent of the caloric needs of the animals and indicate that phospholipid formation is probably not an obligatory step in fat oxidation or transfer.

Rats receiving diets widely divergent in protein, carbohydrate, and fat content may show corresponding divergence in the concentration of phospholipid in the liver, however, if the altered size of the liver is considered, it is found that the formation of new phospholipid for each whole liver is very similar. A deficiency of dietary choline does not greatly reduce the amount of new phospholipid formation, but administration of choline will increase new phospholipid formation. Damage to the liver by carbon tetrachloride does not reduce phospholipid formation. Choline administration may increase phospholipid formation after injury to the liver.

*By Merck & Company, Inc., New York, N. Y.

SEQUELAE OF TRENCH FOOT AND THEIR TREATMENT BY LUMBAR SYMPATHECTOMY

MAJOR DAVID I. ABRAMSON AND LIEUTENANT COLONEL HARRIS B. SHUMACKER, JR. (BY INVITATION), GALESBURG, ILL.

The clinical picture of trench foot, from four to thirteen months after exposure, was studied in a series of 567 patients. The sequelae were divided into three categories.

In the first group, the findings were primarily the result of excessive sympathetic tonus. They consisted of a low cutaneous temperature of the toes, hyperhydrosis, and cyanosis, which at times was replaced by rubor, mottling, and a subjective sensation of coldness of the feet on exposure to a cool environment.

In two patients in whom hyperhydrosis was so marked that maceration of the skin followed by secondary infection resulted, sympathectomy produced an immediate therapeutic effect. The infection cleared up shortly after the cessation of sweating. The operation was also performed in two individuals with cold, blue, wet feet, who complained of pain on exposure to a cold environment. The feet became red and dry, and the marked responsiveness to cold was no longer present.

In the second group, the findings were suggestive of some type of peripheral nerve involvement. They consisted of tenderness of the sole of the foot, resulting in abnormal gaits, hypesthesia, paresthesia, and numbness.

In ten patients of this group, sympathectomy was done in order to treat the plantar tenderness. In six of these, little alteration in symptoms was produced, while in four, slight or moderate relief of pain was noted. Such complaints as paresthesia, hypesthesia, and numbness were unaffected.

The third group showed signs both of excessive sympathetic tone and peripheral nerve involvement. Besides these, desquamation of the plantar surface of the foot, swelling and stiffness of the toes, osteoporosis, and atrophy of the small mucles of the feet were fairly common findings. Sympathectomy was not attempted in the treatment of any of these manifestations.

In the case of gangrene of toes or a portion of the foot, sympathectomy was performed as a preliminary step in twenty-six patients.

It is concluded that this procedure is indicated in the treatment of hyperhydrosis complicated by secondary infection and infrequently in the case of the patient who is incapacitated by a cold, wet, cyanotic foot which is markedly responsive to a cold environment. It also appears to increase the rate of healing of the stump after removal of gangrenous material. Only on occasion does it have any therapeutic effect on the neuritic symptoms of trench foot.

DISCUSSION

DR. GEZA DE TAKATS, Chicago, Ill.—One is beginning to see trench foot in civilian practice and in the veterans' facilities. I fully agree with Major Abramson that the vasomotor group is benefited by sympathectomy, and obviously patients with dysesthesia of the feet will not respond. Nevertheless, occasionally a patient will present himself whose burning pain resembles the causalgic state and in whom sympathetic block abolishes the burning paresthesia.

Such patients are also seen in air hammer disease and unquestionably suffer from demyelinization of some of the sensory fibers. Sympathectomy in such a patient will not relieve the true sensory defect but seems to abolish the sensory stimulation initiated by sympathetic fibers in the demyelinated segments. My experience with a considerable number of cases at the Veterans Hospital would indicate that the majority of patients suffering from trench foot do not require sympathectomy.

DR. JOSEPH EDWARDS, St. Louis, Mo.—In our experience in Italy in 1943 and 1944 at the Twenty-first General Hospital, we had about 350 patients with trench foot. Because of the severity of these cases, it was thought wise to do sympathectomy in a certain selected group. We obtained permission of the theater consultant surgeon, Colonel Churchill, for this work. Accordingly, unilateral sympathectomy was done by Lieutenant Colonels H. G. Schwartz and F. A. Simeone, on thirteen patients most of whom had causalgia, coldness of the feet, and cyanosis. Our conclusions were the same as Major Abramson and Lieutenant Harris B. Shumacker's, that in certain selected patients it seems to be beneficial. We thought only of very severe cold injury with hyperhydrosis often complicated by infection. In some cases of early gangrene, if seen early enough, there may be benefit from sympathectomy. It was of definite benefit in patients with epidermophytosis. We reported some of this work in the United States Army Medical Bulletin. We had occasion to follow these patients four months only. We hope to follow this work by going over the records of these men after they return to the states.

MAJOR ABRAMSON (closing).—With respect to Doctor de Takats' discussion, I cannot add anything further to what I have already stated. There is only an occasional individual with trench foot in whom sympathectomy is followed by a loss of the symptoms of nerve irritation. It is of interest that two of the men who demonstrated marked sensitivity of the soles of the feet also had similar complaints in the heels, and it was the symptoms in the heels which disappeared following operation.

In response to Doctor Edwards' statements, we have seen patients in whom sympathectomy had been performed five, six, or seven months before coming to our hospital, and little difference was noted between the denervated limb and the control with respect to the severity of the response to changes in environment. In fact, some of these patients complained more in the sympathetcomized extremity than in the normally innervated one, but the explanation may be that the sympathectomized extremity was the worse one originally.

TOXIC MANIFESTATIONS FOLLOWING LARGE DOSES OF ERTRON

SMITH FREEMAN, M.D. (BY INVITATION), PAUL S. RHOADS, M.D., AND LEONA B. YAEGER, M.D. (BY INVITATION), CHICAGO, ILL.

Two case histories are presented in which the following findings were similar: ingestion of large doses of Ertron and high milk consumption over long periods followed by extreme weakness, restlessness, subnormal body temperature, weight loss, anorexia, generalized aching, dermatitis, inflammation, and small fatty deposits of the sclerae. Both patients had disturbed calcium metabolism, increased nitrogen retention in the blood, reduced renal function, anemia with eosinophilia, albumin, and casts in the urine. In one patient there was extensive calcification in many bursae and exfoliative dermatitis.

Many of these findings are known to occur in animals poisoned with vitamin D.

In view of the large numbers of persons now being treated with ertron and other high potency vitamin D preparations, a careful check on the complete therapeutic history of patients with the mentioned symptoms is in order. Also

all physicians treating patients with large doses of ertron and other high potency vitamin D preparations should keep a careful check on the urinary findings and calcium-phosphorus value of the blood in addition to insisting on a low calcium diet.

DISCUSSION

DR. ELMER SEVRINGHAUS, Madison, Wis.—This syndrome from the administration of large doses of vitamin D is not limited to ertron and it is not limited to the treatment of chronic arthritis. I doubt that it is caused by large intakes of milk. Two cases were reported from the New Haven Hospital, and I have had one case. Large doses of vitamin D are very helpful in tetany. I have used doses of the magnitude reported without encountering any difficulty. Recently, I have seen one such patient under treatment with 100,000 units of Viosterol who developed the same subjective picture, except that there were no skin manifestations. There were anemia and reduced renal function, clouding of the consciousness, and a peculiar nervousness. Withdrawal of vitamin D with the liberal use of saline administered intravenously brought the serum calcium down from 17 mg. per 100 c.c. of blood, which is a dangerous level. I should say that doses of 50,000 units of vitamin D and upward might be followed by toxic symptoms.

DR. FREEMAN (closing).—It has been shown, for example, that a transient hypercalcemia may be produced by calcium taken orally; however, a more marked and prolonged hypercalcemia will result from the same amount of ingested calcium if the animal has previously received large doses of vitamin D. It has never been possible to dissociate the toxic effects of vitamin D from its influence upon calcium metabolism. For three or four years, I have followed the serum calcium values in several patients with hypoparathyroidism receiving large doses of vitamin D. Such patients may go along all right for months on a given dose of vitamin D and a certain calcium intake, and then for no apparent reason they may develop a hypercalcemia accompanied by symptoms of toxicity. This may be due to an accumulative effect from vitamin D. The prolonged action of vitamin D has been shown in the treatment of rickets and osteomalacia as well as by toxicity studies. Large doses of vitamin D may influence calcium metabolism for months after the vitamin has been discontinued. It is not possible to predict from one patient to the next what dose of sterol will be required to correct a hypocalcemia of a certain magnitude. The requirements of each patient must be determined individually. There is no substitute for serum calcium determinations, and these should always be done in duplicate.

Both the clinical and experimental evidence indicates that a high calcium intake increases the likelihood of toxic manifestations from large doses of vitamin D. Conversely, proper selection of diet and calcium intake will minimize the vitamin D required to correct a hypocalcemia.

THE EFECT OF INFECTION AND TRAUMA ON THE EXCRETION OF URINARY CORTIN*

R. A. SHIPLEY, M.D., AND R. I. DORFMAN, PH.D., CLEVELAND, OHIO

Urinary cortin may be satisfactorily extracted with ethylene dichloride and partially purified by the extraction of inactive material with sodium hydroxide. A cold test modified from Selye and Schenker and a liver glycogen test as used by Dobriner and co-workers are both sufficiently sensitive for the quantitative estimation of urinary cortin. Groups of animals employed in the cold test must be controlled for sensitivity by including a group which receives a standard

*Work done under contract with the Office of Scientific Research and Development.

dose of active adrenal cortical material as well as a group which receives only the solvent. The cold test is potentially four times as sensitive as the glycogen test and thus requires less urine for an assay. However, satisfactory cold test assays may be obtained in only two of three attempts due to variations in sensitivity of animals as a group. Results are more reproducible with the glycogen test. Both tests (assuming the use only of highly sensitive animals in the case of the cold test) give errors of estimate within the range usually encountered in bio-assay procedures. In the case of the glycogen test the error factor (with ten animals in a group receiving the unknown, and $p = 0.05$) is 1.6. This means that the chances are one in twenty that the true value will not vary by more than 1.6 or 1/1.6 times the observed result.

In a series of nineteen normal young men and women the daily excretion of urinary cortin as estimated by the cold test averaged 1.1 mg. of compound A equivalent and varied from 0.5 to 1.8 mg. The glycogen test gave values ranging from less than 0.2 to 0.8 mg. There was no obvious difference between men and women. A sample of pooled normal male urine contained 2.0 mg. compound A equivalent by the cold test and 0.4 mg. by the glycogen test. The disparity between values obtained by the two tests might be attributable to the presence in urine of cortical compounds less active in carbohydrate metabolism than against exposure to cold.

Eighty patients with infectious disease were studied, two after herniorrhaphy and three after extensive body burns. In all three categories there was usually a rise in the output of cortin. The augmentation was high as eightfold in some instances. A patient with a clinical diagnosis of Waterhouse-Friderichsen syndrome excreted appreciable amounts of active material. The postoperative rise occurred during the first two days after operation. During recovery from the mentioned types of stress, there were no instances of an abnormal depression in output which one would expect in the presence of adrenal exhaustion.

THE EFFECT OF DIET ON THE GROWTH AND SURVIVAL OF ADRENALECTOMIZED RATS TREATED WITH DESOXYCORTICOSTERONE ACETATE PELLETS*

ALBERT SEGALOFF, M.D., NEW ORLEANS, LA.
(INTRODUCED BY THOMAS FINDLEY, M.D.)

Immature, adrenalectomized, male rats with subcutaneous pellets of desoxycorticosterone acetate were fed various diets. None of the diets contained any carbohydrate, but all had adequate amounts of the vitamins required for growth in the rat (thiamine chloride, riboflavin, calcium pantothenate, nicotinic acid, pyridoxine, choline chloride, α-tocopherol, vitamin A, and vitamin D_3) and U.S.P. XII salt mixture. When the diet contined crude casein and a fatty acid mixture in addition to the vitamins and salts, all the animals survived and grew for eight weeks, at which time the pellets were removed. However, if the fatty

*This work was done in the Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tenn.

acids were omitted, or vitamin-free casein was substituted for the crude casein, then the animals neither survived very long nor grew. A total of seventy-eight rats were employed on the four diets.

These preliminary studies indicate that (1) dietary carbohydrate is not essential for the growth and survival of adrenalectomized rats maintained on desoxycorticosterone acetate; (2) dietary fatty acids either burn as carbohydrate or permit the utilization of protein by such rats; and (3) there is some substance different from the vitamins required for growth in the rat which is present in crude casein and is required by our adrenalectomized rats.

DISCUSSION

DR. H. T. RICKETTS, Chicago, Ill.—I should like to ask whether any determination of liver function was made on these animals.

DR. SEGALOFF (closing).—We were not able to do liver function tests. The blood sugars were very low in the animals that died and normal in the animals that survived.

CHANGES IN THE PROMINENCE OF THE EYES IN VARIOUS THYROID STATES

BROWN M. DOBYNS, M.D. (BY INVITATION), AND S. F. HAINES, M.D.
ROCHESTER, MINN.

Repeated exophthalmometer measurements of the eyes were made in various types of thyroid disease during the course of treatment.

Thyroidectomy in patients with exophthalmic goiter was usually followed by increased prominence of the eyes. If hypothyroidism supervened, the prominence of the eyes usually increased still further. Treatment of postoperative myxedema usually resulted in regression of the prominence of the eyes to degrees that existed before myxedema developed. Treatment of spontaneous myxedema also resulted in lessening of the prominence of the eyes. Patients with exophthalmic goiter who were treated with thiouracil showed an increase in prominence of the eyes simultaneous with the drop in basal metabolic rate. In most of these instances, the change in the position of the globe was so slight that it could not be detected without measurements. In one patient with severe exophthalmos, the protrusion of the eyes and edema of the lids and of the conjunctivae reached serious proportions during the course of treatment with thiouracil.

DISCUSSION

DR. E. L. DEGOWIN, Iowa City, Iowa.—I should like to reinforce the conclusions of the speakers by reporting the case of a patient whom I recently had a chance to study. This woman is now 61 years old. In 1942 she consulted her local physician because of symptoms which led him to make a diagnosis of myxedema. The laboratory work and basal metabolic rate determinations were very well done. She had all the clinical signs of myxedema, and I think there is no question that she had the condition at that time. Her attending physician started administering thyroid extract; she made a prompt recovery from the symptoms of myxedema and the basal metabolic rate was restored to normal. She has been on thyroid extract in sufficient doses ever since. About six months after beginning therapy with thyroid extract, the doctor noticed that she was developing exophthalmos. This exophthalmos has

continued to progress until the present time without any signs of thyrotoxicosis. Simultaneously with the development of the exophthalmos, she developed localized areas of solid nonpitting edema which many dermatologists, and particularly Dr. Paul O'Leary of the Mayo Clinic, have described in association with exophthalmic goiter. When she came to the hospital, the paradox of a patient with marked exophthalmos and known myxedema intrigued us, and we immediately began to investigate the pituitary. She was then beginning to have a loss of the lower half of both visual fields, and x-ray examination of the skull showed a sclerotic process in the sphenoid bone involving the anterior clinoid process which the radiologist considered most likely to be due to a tumor in that vicinity. Here is a patient who has a tumor in the region of the anterior lobe of the pituitary gland, who first developed signs of thyroid deficiency, and who has been known to develop exophthalmos while receiving adequate doses of thyroid extract. I certainly think that this also brings into question the hypothesis which apparently has been accepted, that malignant exophthalmos is due to the overproduction of thyrotropic hormones as a result of insufficient thyroid hormone.

DR. E. PERRY MCCULLAGH, Cleveland, Ohio.—In observing large numbers of patients with exophthalmic goiter over a period of many years and in making attempts to estimate the factor which may be influencing the exophthalmos, I have been particularly impressed with the great difficulty of establishing any type of control for comparison. Exophthalmos may appear in apparent complete dissociation from evidences of overactivity of the thyroid. For instance, it may make its appearance several years before hyperthyroidism is present, and we have seen it appear and become severe for as long as fourteen years after the disappearance of hyperthyroidism and in the absence of either hyper- or hypometabolism. In view of such facts as these, it is a matter of the greatest difficulty to evaluate the effects of any treatment which is given in an attempt to influence exophthalmos.

There is, I believe, considerable reason to doubt the validity of the current belief that thyrotropic hormone is the cause of progressive exophthalmos. If thyrotropic hormone were actually the cause of exophthalmos, it would seem reasonable to expect that exophthalmos would be present in cases of acromegaly with hyperthyroidism. In such patients, there is a pituitary tumor and goiter with hyperthyroidism, and it seems entirely reasonable to suspect that an excess of thyrotropic hormone must be present, and yet no exophthalmos occurs under these circumstances. Patients with spontaneous myxedema also have an excess of thyrotropic hormone, and these patients do not develop exophthalmos. During thiouracil therapy, there is almost certainly a marked increase in thyrotropic hormone production, and as far as an overall experience with thiouracil has been concerned, there is little indication that exophthalmos usually increases during such treatment. In short, it appears that the exophthalmos of Graves' disease is independent of all the influences which we know of including the effect of thyroid. It is quite evident that hypothyroidism itself does not cure exophthalmos, and it seems to me from this evidence alone that it does not seem reasonable to suppose that the feeding of thyroid would be of much benefit. It seems possible that a more adequate means of inhibiting the pituitary than has been used up to the present may be shown to be the most efficient treatment.

THE CLINICAL SIGNIFICANCE OF THE FUNCTIONAL BEHAVIOR OF ADENOMAS OF THE THYROID GLAND

I. DARIN PUPPEL, M.D., CHARLES P. LEBLOND, M.D. (BY INVITATION), ELSIE RILEY, M.S. (BY INVITATION), AND GEORGE M. CURTIS, M.D., COLUMBUS, OHIO

The exact function of the thyroid nodule or adenoma has not been completely established for the reason that there has been no adequate method to determine the functional activity of various parts of a nodular goiter.

Recently, radio-iodine fractionation study of many forms of pathologic thyroid tissue removed from seven patients with varied clinical types of goiter

and varying degrees of toxicity has given us an unusual opportunity to investigate this problem. These data indicated that the cells of all types of nodular or adenomatous thyroid tissue of nontoxic nodular goiter, toxic nodular goiter, exophthalmic goiter, and so-called fetal adenoma studied are functionally autonomous and consistently showed less avidity for iodine and produced less thyroxine and diiodotyrosine than the groups of thyroid cells in the surrounding thyroid tissue. None could be considered so-called hyperfunctioning adenomas.

The diffuse goitrous tissue of two patients with nontoxic diffuse colloid goiter showed great avidity for iodine, but its ability to produce thyroxine and diiodotyrosine did not appear greatly increased. The diffuse hyperplastic tissue of a patient with exophthalmic goiter showed the greatest avidity for iodine and the greatest ability to produce diiodotyrosine and thyroxine.

The paranodular tissue (that is the thyroid tissue surrounding the nodules) of three patients with nontoxic nodular goiter, one having a mild hypothyroidism, acted biochemically to a greater extent like the tissue of nontoxic colloid goiter. The paranodular tissue of a patient with toxic nodular goiter acted biochemically to a greater extent like the tissue of exophthalmic goiter.

THE EFFECT OF SALICYLATE ON THE SEDIMENTATION RATE, FEVER, AND OCCURRENCE OF VALVULAR HEART DISEASE IN ACUTE RHEUMATIC FEVER

MAJOR HARRY A. WARREN (BY INVITATION), LIEUTENANT COLONEL C. S. HIGLEY, AND MAJOR F. S. COOMBS, JR., MADISON, WIS.

Sodium salicylate has been used in the treatment of acute rheumatic fever for many years. In 1943 Coburn reported a series of thirty-eight patients with acute rheumatic fever, treated with from 10 to 14 Gm. of salicylate daily, in whom valvular heart disease did not develop. Plasma salicylate levels in these patients were found to range above 35 mg. per 100 c.c. In another series Coburn gave smaller amounts of salicylates and obtained lesser plasma values. In twenty-one of sixty-three of these patients, valvular heart disease occurred. We have been interested in the efficacy of salicylate in the treatment of acute rheumatic fever since publication of Coburn's work.

From November, 1942, to September, 1945, we have studied 186 patients with rheumatic fever. Fifty of these patients were given from 10 to 16 Gm. of salicylate daily, orally, until the sedimentation rate remained normal; another forty-eight were given from 10 to 20 Gm. intravenously daily, for seven days, followed by from 10 to 16 Gm. daily, orally; and eighty-eight patients received less than 50 Gm. per week during the acute phase only. Oral or intravenous dosage of 10 Gm. or more per day gave plasma levels in the therapeutic range advocated by Coburn.

We have arbitrarily divided our cases into two groups, severe or moderate, depending upon the initial sedimentation rate. Above 60 mm. per hour was considered evidence of severe disease, while below that figure was considered moderate.

One hundred twenty-six cases fell in the severe category. Forty-five of these patients received the large oral dose of salicylate and required an average of 51.3 days for the sedimentation rate to reach the normal level; forty-four patients received intravenous and then oral salicylate in large doses and required an average of 61.4 days for the sedimentation rate to reach normal; and thirty-seven patients receiving the small oral dosage required 58.7 days for the sedimentation to become normal.

Of the sixty patients in the moderate group, five receiving large oral doses of salicylate required an average of thirty-six days for the sedimentation rate to become normal; four given intravenous and then oral salicylate required an average of 27.75 days for the sedimentation rate to become normal; and fifty-one given small doses of salicylate orally required 35.2 days for the test to become normal.

When all cases are considered, eighty-eight patients receiving small doses of salicylate required an average of forty-five days to reach a normal sedimentation rate. Fifty patients receiving large oral doses required an average of 49.8 days, and forty-eight patients receiving intravenous sodium salicylate required 58.6 days to reach a normal sedimentation rate. When all patients receiving large doses are considered, the average time to reach a normal sedimentation rate was 54.1 days.

Eighty-two patients with a febrile response above 99.2° F. received small doses and required an average of 11.6 days to maintain a normal temperature curve. Eighty-nine patients receiving large doses, either by mouth or by vein, required an average of 4.16 days to maintain normal temperature.

Thirty-five cases, or 39.8 per cent, of those receiving small doses showed a rise in sedimentation rate after reaching a normal level, either with or without clinical signs and symptoms of recurrence. Of those receiving large doses, 61.3 per cent showed such polycyclic courses.

Attempts to determine the presence of organic heart disease were difficult because of the short period of observation. However, seven patients in each group developed significant cardiac murmurs not present on admission or showed definite progressive changes in cardiac abnormalities present on admission.

From these findings, it is apparent that the administration of large doses of salicylates does not reduce the time necessary to reach a normal sedimentation rate and does not protect against the occurrence of polycyclic rheumatic fever. It does eliminate the temperature elevation sooner than small doses. It does not prevent the occurrence of valvular heart disease or prevent the progression of pre-existing cardiac damage to any greater extent than small doses.

DISCUSSION

DR. M. J. SHAPIRO, Minneapolis, Minn.—This report, together with other recent work, shows what has been known for many years: namely, that the salicylates have a good

clinical but no specific effect on rheumatic fever, that the salicylates are rapidly absorbed from the stomach, and that intravenous salicylate therapy is unnecessary and dangerous. Soon after Coburn reported on the use of large doses of sodium salicylate given intravenously, a number of deaths resulted from such therapy.

A word should be said about the use of the sedimentation rate in rheumatic fever as well as about its general use. Not infrequently patients who have apparently recovered from an attack of rheumatic fever continue to have an increased sedimentation rate for weeks and months after all other evidence of activity has disappeared. Such patients are often kept in bed for months for no other reason. Many patients, especially children, have an upper respiratory infection accompanied by joint pains. Such children are frequently kept in bed for long periods of time only because the sedimentation rate is moderately increased. It must be true that many such patients who probably never had rheumatic fever are among those who have been treated prophylactically with the sulfonamides.

If a few of Major Warren's patients were among those who continued to have increased sedimentation rates even though they had recovered from an attack of rheumatic fever, the results of his study would be entirely different.

It is time that the whole matter of the sedimentation rate be restudied. It is quite evident that the increased sedimentation rate alone should not be used as a sole criterion for determining when a patient has recovered from an attack of rheumatic fever.

PENICILLIN AND DICUMAROL, AND PENICILLIN IN THE TREATMENT OF SUBACUTE BACTERIAL ENDOCARDITIS

OVID O. MEYER, M.D., AND CHARLES J. THILL, M.D. (BY INVITATION)
MADISON, WIS.

In June of 1944, it was widely held that penicillin was not efficacious in the treatment of subacute bacterial endocarditis. In January of that year, however, Loewe and his associates reported success in the treatment of this condition with combined penicillin and heparin administration. When penicillin became readily available, in May of 1944, we decided to treat bacterial endocarditis with penicillin and the anticoagulant Dicumarol, a potent cheap drug which can be given orally. The hazards of anticoagulant therapy in this condition were recognized.

Eleven patients have been treated with penicillin and dicumarol. The penicillin dosage ranged from 200,000 units daily given intramuscularly, usually at three-hour intervals, to 1,000,000 units given continuously intramuscularly or intravenously. Dicumarol was given in initial doses of 4 or 5 mg. per kilogram with subsequent daily doses of 1.5 mg. per kilogram as necessary to maintain the prothrombin time between 25 and 50 per cent of normal.

Seven patients recovered and have remained well for intervals of from seven to fourteen months. One patient died after a few days of therapy, possibly from cerebral hemorrhage. Two patients relapsed promptly after completion of therapy, but one of these left the hospital against our wishes before the minimum six-week course, which we have always advocated, was completed. A fourth patient was never brought under control, even with large doses of penicillin and intermittent dicumarol for more than six months. The first patient treated after being well for almost a year had a tooth with a periapical abscess extracted. Prior to the extraction the patient received 15,000 units of penicillin every three hours for five doses and after the operation, the same

amount at the same intervals for fifty-four hours. Within a week he again had positive blood cultures and fever.

Seven patients received penicillin alone in similar doses. One with inadequate therapy, a total of 800,000 units, died of cardiac decompensation two months later, and the vegetations were almost completely healed. Another has been well for a year. She received no dicumarol because of vaginal bleeding present at the time of admission. The other five patients did not respond to treatment, and four have died. The status of the fifth is unknown at present. In these cases, dicumarol was not given because negative blood cultures and control of fever were not attainable, and, after our early experience with the patient who died, we have withheld dicumarol until either a negative blood culture is obtained or the fever controlled. The differences in the results in the two groups are not as important as they seem; therefore, since after the first four patients, only those who were responsive to penicillin received dicumarol. Of the total eighteen patients, eight have recovered, and one of these has again acquired the disease. From our experience to date, we have acquired certain impressions and tentative conclusions.

1. Treatment with penicillin alone or where combined with dicumarol should be persisted for a minimum of six weeks.

2. This therapy has given decidedly better results than anything previously employed.

3. Despite our results favoring combined therapy, we remain decidedly skeptical that anticoagulant therapy offers any advantages, and it is unquestionable that anticoagulant therapy carries with it grave hazards in subacute bacterial endocarditis.

4. Penicillin therapy for fifty-four hours after removal of a focus of infection is *inadequate* to prevent the development of subacute bacterial endocarditis.

5. Seven of our patients who were not cured had received distinctly inadequate therapy before coming under observation, whereas only one of these eight who did recover had had any penicillin prior to ours. All but one of the inadequately treated patients had become subjectively and objectively improved under the original penicillin therapy. We raise the question as to the possible great significance of this point. Although a conclusion regarding it is not justified, it may be important, and an explanation is as yet wanting since in all but one of these patients, the organism was still sensitive in vitro.

DISCUSSION

DR. W. S. MIDDLETON, Madison, Wis.—The introduction of penicillin in the treatment of subacute bacterial endocarditis is a tremendous advance. I have lived through an unwholesome record of 126 consecutive deaths from subacute bacterial endocarditis. I am not boasting but am merely stating this as an experience of the past. In the experience of most workers in this field the addition of an anticoagulant added nothing to the penicillin in the treatment of acute bacterial endocarditis. The route and dosage will be subject to further study. Just one small detail will be called to your attention. The continuous administration of penicillin, either intramuscularly or intravenously, presupposes the use of tubing. At the Medical

Research Council in Great Britain there was reported a reduction of the potency of penicillin passing through tubing other than Latex by about 50 per cent. In other words, if you are going to employ a continuous method of administration, do not use red or black rubber tubing, use latex.

DR. L. N. KATZ, Chicago, Ill.—It is interesting that the report of the Madison group is in agreement with the experience we have had at the Michael Reese Hospital, a preliminary report of which was made to this Society last year.

We have treated seventeen unselected successive patients with penicillin alone (without any combined anticoagulant). Of these seventeen, fourteen were "cured"—some have remained well and at work for over a year. Two patients died during therapy, one from a cerebral hemorrhage and the other from heart failure confirmed at necropsy. Of the other fifteen patients, only one was a treatment failure.

We gave as much as 158 million units of penicillin for as long as eight weeks. Ordinarly we gave from 200,000 to 400,000 units per day for from three to six weeks. We treated one patient with a resistant case with 2,500,000 million units daily toward the end of his course, and on the last three days we gave him para-amino hippuric acid to raise the blood level further. This patient has now recovered and is quite well and at work. One of the recovered patients died of heart failure eight months after therapy was finished. Necropsy confirmed the presence of a healing lesion.

The statistics in the literature and our own experience demonstrate that the addition of anticoagulants is without value. The rate of recovery is as high with penicillin alone as with penicillin and a anticoagulant, when penicillin is administered in large enough dosage for a long enough time, provided that the blood level is maintained high continuously. We used intermittent intramuscular injections given every hour, twenty-four hours a day. Penicillin is excreted very quickly and injections even two hours apart are not adequate to maintain a continuous high blood level. With the large doses we use continuous therapy. This must be maintained constantly day and night throughout the period of treatment.

I think the problem that remains in subacute bacterial endocarditis is the treatment of the penicillin-resistant organisms. In the course of time it may turn out that organisms will be found so resistant that penicillin may be ineffective.

DR. MILTON LANDOWNE, Bethesda, Md.—I would like to ask a question in relation to repeated courses of treatment with penicillin. In the first patient who had been well for almost a year, and in whom bacteremia developed a week after dental extraction despite penicillin, did the blood cultures show the same organism that had been present before? If so, did it have the same degree of in vitro sensitivity to penicillin as it did before? And bearing on this same point, have you any data as to whether the in vitro sensitivity of the organisms to penicillin was altered by the administration of penicillin in the seven patients who, having had previous "inadequate" therapy, failed to respond to your treatment?

DR. HAROLD FEIL, Cleveland, Ohio.—Could treatment be continued in the patient who was sensitive to penicillin?

DR. M. A. BLANKENHORN, Cincinnati, Ohio.—Has Doctor Meyer an opinion on the role of sulfa drugs in resistant penicillin cases?

DR. MEYER (closing).—In the patient who was cured and then relapsed following dental extraction, I cannot tell you whether the organism was identical with that of his first illness. On both occasions, however, the organism was penicillin sensitive and sensitive to 0.30 of a unit per cubic centimeter. It may have been sensitive to a greater dilution, but we did not go below that concentration in testing the sensitivity.

As to the second question, in none of our seven patients do I have information about penicillin sensitivity prior to the time patients came under our observation. Only one was treated by a group where they probably tested the organism for penicillin sensitivity. The second patient was given three million units but did not respond. We could never effect a sterilization of the blood stream.

Answering Doctor Feil's question regarding penicillin sensitivity, we have not observed patient sensitivity to penicillin that required our stopping therapy.

In only one patient was the combination of penicillin and a sulfonamide employed, and without benefit. The dose of penicillin in this case was to 1,000,000 units a day. It does not seem likely that the combination will be especially beneficial, but that is just an opinion.

By May of this year we were fairly convinced that if the patient would not respond to 200,000 units of penicillin a day he would not respond to larger doses. We have certainly changed our minds about that. You will find an occasional patient who will respond to much larger doses than who failed with small doses. We realize that this is not a large series of patients; there are only eighteen. Some of these impressions may not hold, but in 1944 we thought it was a simple matter to cure subacute bacterial endocarditis with penicillin. It is becoming increasingly difficult. We are not getting the same results with the same doses. Perhaps it is pure chance in a small series of cases. Time will tell.

A STUDY OF BERIBERI HEART DISEASE AT THE CINCINNATI GENERAL HOSPITAL

C. F. Vilter, M.D. (By Invitation), M. A. Blankenhorn, M.D., R. S. Austin, M.D. (By Invitation), and I. M. Scheinker, M.D. (By Invitation), Cincinnati, Ohio

In five years at the Cincinnati General Hospital we have recognized beriberi heart disease in twelve patients with cardiac failure. The combined skills of the nutritionist, cardiologist, neurologist, and neuropathologist have been applied to the study of these patients. The following conclusions may be drawn.

Beriberi heart disease in the United States is likely to be missed if one follows strictly the description given by Wenckebach. Although the syndrome of a failing heart with signs of fast circulation occurs and gives a vivid picture, the more common type resembles any other type of degenerative heart disease. Therefore, other criteria have been established to aid in the recognition of beriberi heart disease. Of greatest importance are (1) the elimination of other etiologic agents, (2) a thiamine-deficient diet for longer than three months, (3) the presence of other evidence for nutritional failure, particularly neuritis or pellagra, (4) enlarged heart with sinus rhythm, (5) dependent edema, (6) elevated venous pressure, (7) minor electrocardiographic changes, and (8) recovery with decrease in heart size or necropsy findings consistent with beriberi but not with other types of heart disease.

The onset was sudden in some cases, gradual in others, and healing occurred in the same fashion. The response to thiamine was not dramatic except in one patient. The duration and severity of deficiency probably played a part in the reversibility or irreversibility of the process.

In some instances the condition of the patient was critical at the time of admission. Five of the patients died and necropsy was performed in three of these. In addition to lesions previously reported for beriberi, degenerative changes were found in the central, peripheral, and autonomic nervous systems. The outstanding lesions were (1) degenerative alterations in the cells of the sympathetic nuclei and fibers in the lateral horns of the upper cervical segments of the spinal cord, (2) damage of the spinal cord roots, (3) far-advanced degeneration of the vagus nerves and of some of the peripheral nerve trunks.

These lesions support the theory that the syndrome of beriberi heart disease is associated with neuritis, involving the central and peripheral autonomic nervous systems.

DISCUSSION

DR. EMMET F. PEARSON, Springfield, Ill.—I should like to ask whether blood protein determinations were made on these patients?

DR. GORDON B. MYERS, Detroit, Mich.—In some of our cases of beriberi we have found a sharp inversion of the T wave in Leads V_1 to V_2 which disappeared after thiamine therapy. Have you utilized multiple precordial leads in your cases?

DR. G. E. WAKERLIN, Chicago, Ill.—Were blood pyruvic acid determinations made on these patients? Also was vitamin B complex therapy employed in addition to thiamine?

DR. VILTER (closing).—In each instance there were some blood protein studies; sometimes we measured only the total protein. In practically every instance the serum protein was low. One could not say, however, there was a relative lowering of serum albumin. The total plasma protein was always under 6 and in most instances it was between 4.5 and 5. The dilution factor seemed more important than protein deficiency.

In a few patients we made serial electrocardiographic studies. The electrocardiograms tended to revert to normal as the patient recovered, if he did recover. With progression of the disease there was no progression in the severity of the changes in the electrocardiogram.

We did not make blood pyruvic acid studies in these patients. These studies extended over five years, and we did not use the pyruvic acid level as a routine clinical laboratory test.

We did not use other members of the B complex group in treatment of the usual case. I think that is very important in control studies. In those cases in which it is feasible we like to have first a control period of observation with the patient at rest in bed in order to tell just what bed rest will do, since many previous investigators have simply reported that patients were put to bed and given thiamine. Thiamine itself, as I said before, did not give dramatic results. Bed rest alone may produce moderate improvement. After a suitable period of rest, we gave thiamine alone unless the patient was in a critical stage of nutritive failure. If so, we used ascorbic acid, niacin, and other members of the B complex group. It is wise also to give some natural source of B complex such as brewer's yeast or crude liver extract to these patients since mixed deficiency is so much the rule as to serve as one of the criteria for the diagnosis. Above all, the dietary should be restored and augumented so as to yield from 3,000 to 4,000 calories, 120+ Gm. of protein, and adequate vitamins and minerals.

FOLLOW-UP STUDIES OF PATIENTS WITH CARDIAC WOUNDS

PAUL H. NOTH, M.D., DETROIT, MICH.

This group of patients with penetrating cardiac wounds was restudied for the particular purpose of obtaining electrocardiograms at a late enough period so that the masking effect of pericarditis should have subsided leaving patterns which could be presumed to be due to the wounds themselves. The electrocardiographic findings were compared with other clinical features.

Eight patients were completely examined by me at periods varying between five and thirty-six months and averaging nineteen and four-tenths months following the wounds. Five complained of a variety of indefinite symptoms and three had no symptoms. However, the latter all had abnormal electrocardiograms, whereas three of the five patients with symptoms had normal tracings.

Abnormal physical findings were absent in all except two patients. Venous pressure was normal in the seven patients tested for this.

Ten patients had roentgenologic studies. Roentgenograms were entirely normal in eight. Three of these eight patients had abnormal electrocardiograms. Two patients exhibited abnormal quality of cardiac pulsation as observed fluoroscopically. Both had abnormal tracings.

Twelve patients were followed electrocardiographically for periods averaging eighteen months. All had precordial leads and nine had six precordial leads (Wilson type of central electrode) and augmented unipolar extremity leads. This group is a notable addition to the twenty-three cases of penetrating heart wounds with published electrocardiograms recorded in the literature, without ligation of a coronary artery, which were followed for three months or longer. The average follow-up period was only five and six-tenths months.

In the present study four of the six patients with left ventricular wounds had abnormal electrocardiograms. All showed abnormalities of the T waves in Leads I and V_6, and sometimes also in Leads II, V_4, and V_5. The electrocardiogram of one of the three patients with right ventricular wounds was abnormal and showed incomplete right bundle branch block, right ventricular premature systoles, and T wave abnormalities most marked in Leads V_3 to V_5. The electrocardiogram of a patient whose wound was of the pulmonary vein and pericardium showed changes characteristic of acute pericarditis, apparently due to persisting empyema and complicating pericarditis. Two patients with less severe wounds not requiring operation had normal tracings.

In general, the electrocardiograms exhibit the most persistent objective abnormalities; consequently, they are of value as an indication of the need for some caution in the management of such cases. When the electrocardiogram was normal, other objective studies also were normal and the persisting symptoms strongly suggested a complicating cardiac neurosis. These late electrocardiograms in this series and in the literature confirm the impression that heart wounds in themselves often produce localizing electrocardiographic changes and that these changes are usually seen in the leads reflecting also the common types of involvement of the right or left ventricles. Multiple precordial leads may add useful localizing information.

THE PRECORDIAL ELECTROCARDIOGRAM IN HIGH LATERAL MYOCARDIAL INFARCTION

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Observations have been made in eight patients whose routine standard and precordial electrocardiograms showed only slight or suggestive evidence of myocardial infarction, whereas more extensive exploration of the upper left anterior, lateral and posterolateral aspect of the thorax disclosed more diagnostic changes. Four of the seven patients gave a history of acute coronary thrombosis a few days to one year prior to the observations. Two had moderately severe congestive heart failure and two had severe peripheral vascular disease; in these four patients the time of occurrence of the infarction was less certain.

In five patients the most characteristic changes of myocardial infarction were recorded in the vertical line of V_3 and V_4 but from one to three interspaces higher than the usual level; these we have designated as high anterolateral infarction. In two patients the most marked changes occurred in leads from the anterior or mid-axillary lines but two or three interspaces higher than the usual V_5 or V_6; these we have termed high lateral infarction. In one case the most striking changes were observed in records taken high in the posterior axillary and left scapular lines and were attributed to a high posterolateral infarct. The observations made were not the same in all patients since only as the study progressed were the most advantageous points for exploration revealed.

Some patients with high anterolateral or posterolateral infarction displayed either small Q waves, slightly inverted T waves or both, but others displayed only minor changes in the standard and unipolar left arm (V_L) leads. These leads showed more significant changes in those patients with high lateral infarction, suggesting that the involved area faced the left shoulder more directly. In the entire group of patients the routine precordial leads (V_1 through V_6) either disclosed some diminution in the size of the R waves or failed to show the usual rapid increase in size of the R waves as the exploring electrode was moved from right to left, but prominent Q or QS waves did not appear.

It is suggested that observations of the kind reported here be made, not routinely, but in those instances in which the history, limb leads, or both are suggestive of myocardial infarction and in which the usual precordial leads fail to yield conclusive evidence of this condition.

DISCUSSION

DR. GORDON B. MYERS, Detroit, Mich.—About a year ago we encountered two patients who had diagnostic signs of infarction in Goldberger Lead aV_L with inconclusive findings in other leads. Both went to autopsy and a diagnosis of high lateral myocardial infarction was confirmed. Since that time we have made use of high axillary leads comparable to Doctor Rosenbaum's, but no opportunity for further autopsy confirmation has been offered.

In the interpretation of Goldberger Lead aV_L, one must bear in mind that in vertically placed hearts the mitral orifice may face toward the left arm, resulting in the transmission of the potentials of the left ventricular cavity to the left arm, causing a Q wave and inverted T wave in Lead aV_L. This finding is more common when the electrocardiogram is taken in the recumbent posture and tends to disappear when the electrocardiogram is taken in the sitting position.

ELECTROCARDIOGRAPHIC DIAGNOSIS OF RIGHT VENTRICULAR LESIONS WITH ESPECIAL REFERENCE TO THE FINDINGS IN THE WILSON PRECORDIAL AND GOLDBERGER EXTREMITY LEADS

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This study is based upon twenty cases in which an electrocardiographic diagnosis of right ventricular hypertrophy was confirmed at autopsy and upon forty-one additional cases in which an electrocardiographic diagnosis of "acute

right ventricular strain'' was supported by the post-mortem finding of right ventricular dilatation which was associated with extensive pulmonary infarction, consolidation, or atelectasis in twenty-two of the cases and was secondary to acute left ventricular failure in nineteen. The standard leads, together with $V_{2, 4, 6}$ were taken on all cases; in addition, leads $V_{1, 3, 5}$ and the Goldberger extremity potentials were taken on thirty-two cases. Post-mortem examination included injection of coronary circulation in thirty-two cases; determination of the ratios of the right and left ventricular muscle mass in thirty-eight cases. This series was augmented by a larger group of cases in which the diagnosis rested on clinical grounds.

The electrocardiographic pattern of right ventricular hypertrophy is characterized by a prominent R wave with late intrinsicoid deflection in leads V_1, V_2 and aV_R; a relatively small S wave in $V_{1, 2}$ and relatively large S wave in $V_{5, 6}$; and depression of the RS-T junction with diphasic to inverted T waves in Leads V_1, V_2, and usually V_3. The findings were essentially the same irrespective of whether the right ventricular hypertrophy was secondary to congenital heart disease, mitral stenosis, or chronic pulmonary disease. The electrocardiographic pattern of ''acute right ventricular strain'' was characterized chiefly by the abrupt development of sharply inverted T waves with convex RS-T segments in $V_{1, 2, 3}$ followed by rapid changes in serial curves. ''Right ventricular strain'' was differentiated from anteroseptal infarction by the absence of Q wave pattern and by the fact that the T wave changes were less marked in V_3 than in V_2 and V_1. Right bundle branch block, which may also occur in association with right ventricular hypertrophy or ''acute right ventricular strain,'' was not included in this study.

DISCUSSION

Dr. L. N. Katz, Chicago, Ill.—I think Doctor Myers is to be congratulated upon an analysis of a very difficult field. All of us who have had experience in electrocardiography have come to realize that we cannot readily diagnose right heart strain from the ordinary limb leads. Unless we know clinically that the right heart is enlarged it is often impossible to make the diagnosis from the electrocardiogram alone. Such a diagnosis of right heart strain can be made from the limb leads if S_1 and S_2 are very deep. The diagnosis can often be made from the appearance of the chest leads CF_2, CF_4 and CF_5, especially when the R/S ratio decreases from CF_2 to CF_5. I am still reluctant to believe that the so-called unipolar leads, which are really not unipolar, can be helpful.

Dr. Francis F. Rosenbaum, Ann Arbor, Mich.—I feel that there are some situations in which the findings reported by Doctor Myers and Doctor Stofer may be confusing, particularly in the differentiation of right ventricular hypertrophy and right bundle branch block. This is especially the case when we are dealing with incomplete right bundle branch block. We have not found that the unipolar lead from the right arm (V_R) is of particular help since large R waves are seen in this lead in both conditions. It is true that this R wave is more sharp and peaked in right ventricular hypertrophy, whereas in right bundle branch block it is more likely to be notched, broad, or slurred.

Dr. Myers (closing).—We have had the same difficulty as Doctor Rosenbaum in distinguishing between the classical pattern of right ventricular hypertrophy and right incomplete bundle branch block. However, our cases of incomplete right bundle branch block who have come to autopsy showed right ventricular hypertrophy, so the distinction is mainly of academic interest.

IRREVERSIBLE AND SLOWLY REVERSIBLE CHANGES IN SEVERE MALNUTRITION

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Of the 567 white civilian patients admitted with the primary diagnosis of malnutrition to the United States Army hospitals in Manila after its liberation, many died in spite of efforts to reverse the degenerative processes. Other patients slowly recovered from various physiologic disturbances and in some there was certain evidence of permanent tissue damage.

Among the degenerative processes observed were nerve changes; changes in the retinae, optic nerve, and lens, causing failing vision; evidence of myocardial damage; skeletal muscle weakness; and certain evidence of damage to the viscera.

DISCUSSION

DR. C. F. VILTER, Cincinnati, Ohio.—Doctor Blankenhorn asked me to discuss this paper for him because he had to leave.

I would like to reply to the questioning of thiamine deficiency as the cause of beriberi heart disease in our patients. Although the plasma proteins of every patient were low, it seems obvious that this was due to the dilution factor of cardiac edema rather than to primary protein deficiency. Although most of these patients were on a low protein, thiamine-deficient diet, the plasma proteins were restored to normal coincident with thiamine administration and cardiac compensation, a much more rapid restoration than could be afforded by dietary protein supplements. The single patient, who showed a dramatic cure following thiamine, exemplifies the fact that protein deficiency is not causal in the disorder. This patient had massive edema and the total proteins were 6.4 Gm. per cent. After three days on the control diet, she was given thiamine, and diuresis began in twenty-four hours. In a week she lost twenty-one pounds of edema fluid. Protein intake was low during this period. Studies made in the past by Wenckebach, Weiss, Wilkins, Keefer, and many others have definitely indicated that thiamine deficiency is the cause of, and administration of thiamine the cure for, beriberi heart disease.

I would like to ask if Doctor Pearson has made any studies which determine that protein alone causes remission of beriberi.

DR. PEARSON (closing).—All of the cases I saw that were called "wet" beriberi had hypoproteinemia. That is the only evidence I have to go on. I think Doctor Vilter stated this morning, that his patients with beriberi heart had hypoproteinemia, from 4 to 6 Gm. I believe a finding of 6 Gm. is definite hypoproteinemia. One gram deficiency in the blood represents considerable deficiency in the body in general. I have not made any further studies to determine whether hypoproteinemia with edema and beriberi are the same thing, but if all of a group of patients with beriberi have demonstrable hypoproteinemia, I consider that sufficient evidence. The confusing thing is that most individuals who are forced to remain on a low thiamine diet are also on a low protein diet. These people at Santo Tomas had nothing but a carbohydrate diet. One of the things that may happen when there is a prolonged carbohydrate diet without protein is a loss of the mechanism for digestion of protein. The trypsin is measurably decreased. If the mechanism for protein digestion is not worked regularly, it will cease to be efficient.

THE TOTAL SOLIDS, FAT, AND NITROGEN IN THE FECES OF PERSONS WHO HAVE UNDERGONE PARTIAL GASTRECTOMY WITH ANASTOMOSIS OF THE ENTIRE CUT END OF THE STOMACH TO THE JEJUNUM (POLYA ANASTOMOSIS)

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A small percentage of patients subjected to partial gastrectomy for lesions of the stomach and duodenum experience difficulty in gaining weight after operation. The inability to gain weight appears to be due in part to the reluctance of the patient, because of his distress, to take an adequate diet. However, it seemed possible that inability to gain weight also might be due in part to impaired utilization of ingested food. This possibility led to the following metabolic studies.

A series of thirteen persons who had not undergone gastric surgery were fed a diet containing 208 Gm. of fat, 97.5 Gm. of protein, 140.5 Gm. of carbohydrate and 2,823 calories per day. A second series consisting of ten persons who had undergone partial gastrectomy with anastomosis of the entire cut end of the stomach to the jejunum were fed the same diet. A third series of four persons who had undergone partial gastrectomy with anastomosis of the entire cut end of the stomach to the jejunum also were studied; these, however, were fed weighed diets of lower fat content. These diets contained from 100 to 125 Gm. of fat and from 90 to 100 Gm. of protein. The carmine marker technique was used to begin and to end the period of study. Seventy-two-hour samples of stools were collected from the patients in Groups 1 and 2; longer periods of collection were used in Group 3. The total solids, fat, and nitrogen were measured in all samples.

Analysis of the data obtained disclosed the following:

1. Nearly all persons studied who had undergone partial gastric resection with anastomosis of the entire cut end of the stomach to the jejunum lost more fat in the stool when taking either a diet containing a moderate or a high amount of fat than did those who had not undergone any operation on the stomach. This loss occurred in patients who were not having symptoms referable to the digestive tract but tended to be greater among patients who had postoperative digestive complaints.

2. Some patients with partial gastrectomy and the anastomosis of the type described lost more nitrogen in the stool than did those who had not had an operation on the stomach.

3. In most instances the amount of fat and nitrogen in the feces in excess of normal was not large, but this higher excretion may be one of the causes of inability to gain weight, especially when the intake of food is limited by the patient's postcibal distress.

DISCUSSION

DR. ELMER L. SEVRINGHAUS, Madison, Wis.—I would like to ask if in any of these patients there are any data indicating the value of special types of fat, such as milk fat compared with the other fats in the diet?

DR. G. E. WAKERLIN, Chicago, Ill.—Was the effect of pancreatic enzymic preparations studied in these partially gastrectomized patients?

DR. ROBERT M. STECHER, Cleveland, Ohio.—How many days postoperative were these studies made? Was there a variation with the increase in the time interval after operation?

DR. WOLLAEGER (closing).—In answer to the question concerning the utilization of different kinds of fat, we have tried only one type of diet in which the fat consisted of cream, butter, and meat fat. We have not studied the utilization of other kinds of fat.

We have not done enough work with pancreatic preparations to make any statement about their effect in regard to the utilization of fat by persons who have undergone a gastrectomy. We studied one patient who had a rather large fat loss in the stool and an achlorhydria following the Ewald test meal. He was given large doses of powdered pancreatin during one three-day period of study and the fat loss in the stool was reduced to normal amounts. We have studied no other patient in this manner.

The length of time following operation that our patients with gastrectomy were studied varied from three months to eight years.

THE EFFECT OF ALUMINA GEL UPON THE ABSORPTION OF NUTRIENT SUBSTANCES FROM THE INTESTINAL TRACT

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To study the possible effect upon intestinal absorption of therapeutic doses of alumina gel, tolerance curves were determined in hospital control subjects of vitamin A, ascorbic acid, glucose, fats, and amino acid, first under control conditions and then after the ingestion of alumina gel. In seventeen subjects, the vitamin A tolerance curves, obtained after ingestion of 75,000 I.U., were slightly but significantly depressed if 1 ounce of alumina gel (Amphojel) was given simultaneously. This depression did not occur if alumina gel was given every two hours for one or more weeks before the second tolerance curve was obtained. The depressed absorption (if that was the cause of the flattened curve) was therefore not due to any tanning action of the alumina gel. With aluminum phosphate gel no depression of the vitamin A tolerance curves was seen. That the slight depression of vitamin A tolerance curves has no nutritional significance is indicated by the finding that in thirty-three patients with peptic ulcer who had been on alumina gel management for two or more weeks, the plasma vitamin A and carotenoid concentrations showed no deviation from the normal.

Tolerance curve comparisons were made in twelve subjects for amino acids, in twelve subjects for ascorbic acid, in fifteen subjects for glucose, and in ten subjects for neutral fat. In the first three, statistically insignificant flattening of the tolerance curves occurred; in the case of fat, there was no demonstrable effect of alumina gel. It is possible that alumina gel, by reducing the amount of available phosphate, may interfere slightly with phosphorylation where that process is involved in intestinal absorption.

BOOK REVIEWS

Familial Nonreaginic Food-Allergy. By *Arthur F. Coca,* M.D., Medical Director, Lederle Laboratories. Charles C Thomas, Springfield, Ill., 1945. Price $3.75. Cloth with 185 pages.

Year Book of Industrial and Orthopedic Surgery. Edited by *Charles F. Painter,* M.D., Orthopedic Surgeon to the Massachusetts Women's Hospital and Beth Israel Hospital, Boston, Mass. The Year Book Publishers, Inc., Chicago, Ill., 1946. Price $3.00. Cloth with 432 pages.

Announcement

The Blood Transfusion Association of New York City will resume grants-in-aid for research in the field of blood and blood substitutes in relation to transfusions.

Those interested in obtaining such grants should write, giving full information concerning their projects, to the Chairman of the Research Grants Committee, 2 West 106th Street, New York 25, N. Y.

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